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(54) Title: GENE THERAPY FOR EFFECTOR CELL REGULATION

(57) Abstract

The present invention provides a nucleic acid-based therapeutic composition to treat an animal with disease by controlling the activity of effector cells, including T cells, macrophages, monocytes and/or natural killer cells, in the animal. Therapeutic compositions of the present invention include superantigen-encoding nucleic acid molecules, either in the presence or absence of a cytokine-encoding nucleic acid molecule and/or chemokine-encoding nucleic acid molecules, depending upon the disease being treated. The present invention also relates to an adjuvant for use with nucleic acid-based vaccines. Adjuvant compositions of the present invention include an immunogen combined with superantigen-encoding nucleic acid molecules, either in the presence or absence of a cytokine-encoding nucleic acid molecule and/or chemokine-encoding nucleic acid molecules.

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GENE THERAPY FOR EFFECTOR CELL REGULATION

FIELD OF THE INVENTION

The present invention relates to a product and process for regulating T cell activity by providing a superantigen

5 gene, in the presence or absence of a cytokine and/or chemokine gene. The present invention also relates to a product and process for regulating T cell activity by providing a peptide and a superantigen gene, in the presence or absence of a cytokine and/or chemokine gene. In

10 particular, the present invention relates to a product and process for controlling tumor development, immune responses to infectious diseases and diseases caused by immunological disorders.

BACKGROUND OF THE INVENTION

15

Two major causes of disease include infectious agents and malfunctions of normal biological functions of an animal. Examples of infectious agents include viruses, bacteria, parasites, yeast and other fungi. Examples of

20 abnormal biological function include uncontrolled cell growth, abnormal immune responses and abnormal inflammatory responses. Traditional reagents used attempt to protect an animal from disease include reagents that destroy infectious agents or cells involved in deregulated

25 biological functions. Such reagents, however, can result in unwanted side effects. For example, anti-viral drugs that disrupt the replication of viral DNA also often disrupt DNA replication in normal cells in the treated patient. Other treatments with chemotherapeutic reagents to

destroy cancer cells typically leads to side effects, such as bleeding, vomiting, diarrhea, ulcers, hair loss and increased susceptibility to secondary cancers and infections.

5   An alternative method of disease treatment includes modulating the immune system of a patient to assist the patient's natural defense mechanisms. Traditional reagents and methods used to attempt to regulate an immune response in a patient also result in unwanted side effects and have

10   limited effectiveness. For example, immunosuppressive reagents (e.g., cyclosporin A, azathioprine, and prednisone) used to treat patients with autoimmune disease also suppress the patient's entire immune response, thereby increasing the risk of infection. In addition,

15   immunopharmacological reagents used to treat cancer (e.g., interleukins) are short-lived in the circulation of a patient and are ineffective except in large doses. Due to the medical importance of immune regulation and the inadequacies of existing immunopharmacological reagents,

20   reagents and methods to regulate specific parts of the immune system have been the subject of study for many years.

Stimulation or suppression of the immune response in a patient can be an effective treatment for a wide variety

25   of medical disorders. T lymphocytes (T cells) are one of a variety of distinct cell types involved in an immune response. The activity of T cells is regulated by antigen, presented to a T cell in the context of a major

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histocompatibility complex (MHC) molecule. The T cell receptor (TCR) then binds to the MHC:antigen complex. Once antigen is complexed to MHC, the MHC:antigen complex is bound by a specific TCR on a T cell, thereby altering the
5   activity of that T cell.

The use of certain staphylococcal enterotoxin proteins that are capable of complexing with MHC molecules to influence T cell function has been suggested by various investigators, including, for example, White et al., *Cell*
10   56:27-35, 1989; Rellahan et al. *J. Expt. Med.* 172:1091-1100, 1990; Micusan et al., *Immunology* 5:3-11, 1993; Hermann et al., *Immunology* 5:33-39, 1993; Bhardwaj et al., *J. Expt. Med.* 178:633-642, 1993; and Kalland et al., *Med. Oncol. & Tumor Pharmacother.*, 10:37-47, 1993. In
15   particular, various investigators have suggested that *Staphylococcal enterotoxin* proteins are useful for treating tumors, including Newell et al., *Proc. Natl. Acad. Sci. USA* 88:1074-1078, 1991; Kalland et al., PCT Application No. WO 91/04053, published April 4, 1991; Dohlstein et al., *Proc.*
20   *Natl. Acad. Sci. USA* 88:9287-9291, 1991; Hedlund et al., *Cancer Immunol. Immunother.* 36:89-93, 1993; Lando et al., *Cancer Immunol. Immunother.* 36:223-228, 1993; Lukacs et al., *J. Exp. Med.* 178:343-348, 1993; Ochi et al., *J. Immunol.* 151:3180-3186, 1993; and Terman et al., PCT
25   Application No. WO 93/24136, published December 9, 1993. These investigators, however, have only disclosed the use of bacterial enterotoxin proteins themselves. The use of

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bacterial enterotoxin protein has the major disadvantage of being toxic to the recipient of the protein.

Thus, there is a need for a product and process that allows for the treatment of disease using bacterial
5   enterotoxins in a non-toxic manner.

SUMMARY

Traditional pharmaceutical reagents used to treat cancer, infectious diseases and diseases caused by
10 immunological disorders often have harmful side effects. In addition, such reagents can be unpredictable (e.g., treatment of cancer, vaccination against infectious agents). For example, chemotherapy and radiotherapy often cause extensive normal tissue damage during the process of
15 treating cancerous tissue. In addition, vaccine treatments for the prevention or cure of infectious diseases are often ineffective because adjuvants useful in vaccine therapy are toxic to an animal.

The present invention is particularly advantageous in
20 that it provides an effective therapeutic composition that enables the safe treatment of an animal with a reagent that is a potentially toxic an immunogenic protein. Upon delivery, expression of acid molecules contained in the therapeutic composition result in localized production of
25 an effective but non-toxic amount of encoded proteins that may be toxic at concentrations that would be required if the encoded proteins were administered directly. The therapeutic compositions of the present invention can

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provide long term expression of the encoded proteins at a site in an animal. Such long term expression allows for the maintenance of an effective, but non-toxic, dose of the encoded protein to treat a disease and limits the frequency

5   of administration of the therapeutic composition needed to treat an animal. In addition, because of the lack of toxicity, therapeutic compositions of the present invention can be used in repeated treatments.

10   BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 illustrates the expression of superantigen-encoding DNA plasmids in mammalian cells.

Fig. 2 illustrates the proliferative response of canine PBMC's to canine melanoma cells transfected with a

15   superantigen-encoding DNA plasmids.

Figs. 3A and 3B illustrate the release of superantigen protein by CHO cells transfected with superantigen-encoding DNA plasmids.

Fig. 4 illustrates the proliferative response of the

20   Vβ3+ T cell clone AD10 to melanoma cells transfected with superantigen-encoding DNA plasmid.

Fig. 5 illustrates the release of canine GM-CSF by CHO cells transfected with GM-CSF-encoding DNA plasmid.

Figs. 6A and 6B illustrate the vaccination of mice

25   with autologous tumor cells transfected with superantigen-encoding DNA plasmid.

Fig. 7 illustrates the effect of tumor target transfection on cytotoxic T cell lysis.

Fig. 8 illustrates the response of Vβ3+ T cells to intramuscular injection of a superantigen-encoding DNA plasmid.

Fig. 9 illustrates the antibody response resulting
5   from the co-administration of DNA encoding an adjuvant and DNA encoding ovalbumin.

Fig. 10 illustrates that the co-administration of DNA encoding an adjuvant and DNA encoding ovalbumin increase interferon-gamma release from T cells restimulated *in vitro*
10   by the ovalbumin protein.

Fig. 11 illustrates that the co-administration of DNA encoding an adjuvant and DNA encoding ovalbumin increase T cell proliferative responses to ovalbumin.

Fig. 12 illustrates that the co-administration of DNA
15   encoding an adjuvant and DNA encoding ovalbumin increases CTL responses to ovalbumin.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a novel product and
20   process for controlling effector cell activity.   The present invention also relates to a novel adjuvant useful for enhancing an immune response. It is now known for the first time that a composition containing nucleic acid molecules encoding a superantigen, rather than superantigen
25   proteins, is an effective therapeutic reagent for treating disease and is an effective adjuvant for enhancing an immune response. As used herein, a disease refers to any biological abnormality that is not beneficial to a subject.

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The present inventors have also discovered that administration of a combination of nucleic acid molecules encoding: (1) a superantigen; (2) a superantigen and a cytokine; or (3) a superantigen and a chemokine, can act

5   synergistically to effectively treat cancer and infectious disease.   The present invention includes therapeutic compositions comprising: (a) an isolated nucleic acid molecule encoding a superantigen; or (b) an isolated nucleic acid molecule encoding a superantigen in

10   combination with an isolated nucleic acid molecule encoding a cytokine and/or an isolated nucleic acid molecule encoding a chemokine.   Administration of a therapeutic composition of the present invention to an animal results in the production of superantigen, cytokine or chemokine

15   proteins, referred to herein as "encoded proteins." Each of the components of a therapeutic composition of the present invention is described in detail below, followed by a description of the methods by which the therapeutic composition is used and delivered.

20   One embodiment of the present invention includes a method for increasing effector cell immunity in an animal, the method comprising administering to an animal an effective amount of a therapeutic composition comprising: (a) an isolated nucleic acid molecule encoding a

25   superantigen; or (b) an isolated nucleic acid molecule encoding a superantigen in combination with an isolated nucleic acid molecule encoding a cytokine and/or an isolated nucleic acid molecule encoding chemokine.

According to the present embodiment, the nucleic acid molecules are operatively linked to one or more transcription control sequences and the therapeutic composition is targeted to a site in the animal that contains an abnormal cell. According to the present invention, an effector cell, includes a helper T cell, a cytotoxic T cell, a macrophage, a monocyte and/or a natural killer cell. For example, the method of the present invention can be performed to increase the number of effector cells in an animal that are capable of killing or releasing cytokines or chemokines when presented with antigens derived from an abnormal cell or a pathogen. An effective amount of a therapeutic composition of the present invention comprises an amount capable of treating a disease as described herein. Alternatively, a method of the present invention can be performed to decrease the number of T cells found in a T cell subset that is preferentially stimulated and expanded by an autoantigen.

As used herein, effector cell immunity refers to increasing the number and/or the activity of effector cells in the area of the abnormal cell. In particular, T cell activity refers to increasing the number and/or the activity of T cells in the area of the abnormal cell. Also, as used herein, an abnormal cell refers to a cell displaying abnormal biological function, such as abnormal growth, development or death. Abnormal cells of the present invention, preferably includes cancer cells, cells infected with an infectious agent (i.e., a pathogen) and non-

cancerous cells having abnormal proliferative growth (e.g.,
sarcoidosis, granulomatous disease or papillomas) and with
cancer cells and infected cells. Another embodiment of the
present invention is a method to treat an animal with

5   cancer, the method comprising administering to an animal an
effective amount of a therapeutic composition comprising:
(a) a nucleic acid molecule encoding a superantigen; or (b)
a nucleic acid molecule encoding a superantigen in
combination with an isolated nucleic acid molecule encoding

10   a cytokine and/or a nucleic acid molecule encoding a
chemokine.   According to the present embodiment, the
nucleic acid molecules are operatively linked to one or
more transcription control sequences and the therapeutic
composition is targeted to the site of a cancer.

15   One embodiment of a therapeutic composition of the
present invention comprises an isolated nucleic acid
molecule encoding a superantigen (also referred to herein
as a "superantigen-encoding" nucleic acid molecule).
Another embodiment of a therapeutic composition of the

20   present invention comprises an isolated nucleic acid
molecule encoding a superantigen, combined with an isolated
nucleic acid molecule encoding a cytokine (also referred to
herein as a "cytokine-encoding" nucleic acid molecule)
and/or a nucleic acid molecule encoding a chemokine (also

25   referred to as a "chemokine-encoding" nucleic acid
molecule). According to these embodiments, the nucleic
acid molecules are operatively linked to one or more
transcription control sequences. It is to be noted that

the term "a" or "an" entity refers to one or more of that entity; for example, a compound refers to one or more compounds. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein.

5   According to the present invention, an isolated, or biologically pure, nucleic acid molecule, is a nucleic acid molecule that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the nucleic acid molecule has

10   been purified.   An isolated nucleic acid molecule can include DNA, RNA, or derivatives of either DNA or RNA. An isolated superantigen or cytokine nucleic acid molecule can be obtained from its natural source, either as an entire (i.e., complete) gene or a portion thereof capable of

15   encoding a superantigen protein capable of binding to an MHC molecule or a cytokine protein capable of binding to a complementary cytokine receptor. A nucleic acid molecule can also be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification,

20   cloning) or chemical synthesis. Nucleic acid molecules include natural nucleic acid molecules and homologues thereof, including, but not limited to, natural allelic variants and modified nucleic acid molecules in which nucleotides have been inserted, deleted, substituted,

25   and/or inverted in such a manner that such modifications do not substantially interfere with the nucleic acid molecule's ability to encode a functional superantigen or a functional cytokine of the present invention.

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A nucleic acid molecule homologue can be produced using a number of methods known to those skilled in the art (see, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989).

5   For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis techniques and recombinant DNA techniques, such as site-directed mutagenesis, chemical treatment of a nucleic acid molecule to induce mutations,

10 restriction enzyme cleavage of a nucleic acid fragment, ligation of nucleic acid fragments, polymerase chain reaction (PCR) amplification and/or mutagenesis of selected regions of a nucleic acid sequence, synthesis of oligonucleotide mixtures and ligation of mixture groups to

15 "build" a mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologues can be selected from a mixture of modified nucleic acids by screening for the function of the protein encoded by the nucleic acid (e.g., superantigen, cytokine or chemokine

20 activity, as appropriate). Techniques to screen for superantigen, cytokine or chemokine activity are known to those of skill in the art.

Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase

25 "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being

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capable of encoding a superantigen, a cytokine or a chemokine protein.   In addition, the phrase "recombinant molecule" primarily refers to a nucleic acid molecule operatively linked to a transcription control sequence, but

5   can be used interchangeably with the phrase "nucleic acid molecule" which is administered to an animal. As heretofore disclosed, superantigen or cytokine proteins of the present invention include, but are not limited to, proteins having full-length superantigen, cytokine or chemokine coding

10   regions, proteins having partial superantigen regions capable of binding to an MHC molecule, cytokine coding regions capable of binding to a complementary cytokine receptor, chemokine coding regions capable of binding to a complementary chemokine receptor, fusion proteins and

15   chimeric proteins comprising combinations of different superantigens, cytokines and/or chemokines.

One embodiment of the present invention is an isolated superantigen-encoding nucleic acid molecule that encodes at least a portion of a full-length superantigen, or a

20   homologue of a superantigen.   As used herein, "at least a portion of a superantigen" refers to a portion of a superantigen protein capable of binding to an MHC molecule in such a manner that a TCR can bind to the resulting superantigen:MHC complex.   Preferably, a superantigen

25   nucleic acid molecule of the present invention encodes an entire coding region of a superantigen, and more preferably the coding region absent a leader sequence. Production of a truncated superantigen protein lacking a bacterial leader

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sequence is preferred to enhance secretion of the superantigen from a cell. As used herein, a homologue of a superantigen is a protein having an amino acid sequence that is sufficiently similar to a natural superantigen amino acid sequence that a nucleic acid sequence encoding the homologue encodes a protein capable of binding to an MHC molecule.

In accordance with the present invention, a superantigen comprises a family of T cell regulatory proteins that are capable of binding both to an MHC molecule. A superantigen binds to the extracellular portion of an MHC molecule to form and MHC:superantigen complex. The activity of a T cell can be modified when a TCR binds to an MHC:superantigen complex. Under certain circumstances, an MHC:superantigen complex can have a mitogenic role (i.e., the ability to stimulate the proliferation of T cells) or a suppressive role (i.e., deletion of T cell subsets). The ability of an MHC:superantigen complex to have a stimulatory or suppressive role can depend upon factors, such as the concentration and environment (i.e., tissue location and/or the presence of cytokines).

The mitogenic role of a superantigen is distinct from other known mitogens (e.g., lectins derived from plants) in that superantigens are capable of stimulating the proliferation of particular subsets of T cells having TCR's that specifically bind to the superantigen. For example, a superantigen, when added to a mixed lymphocyte

population, is able to stimulate the proliferation of a select population of T cells from the mixed population of cells. Examples of T cell subsets stimulated by superantigens complexed with MHC molecules include T cells

5   expressing a TCR comprising mouse $V_\beta 1$, $V_\beta 3$, $V_\beta 7$, $V_\beta 8.1$, $V_\beta 8.2$, $V_\beta 8.3$, $V_\beta 10$, $V_\beta 11$, $V_\beta 17$, $V_\beta 15$ or $V_\beta 16$ chains, and T cells expressing a TCR comprising human $V_\beta 1.1$, $V_\beta 2$, $V_\beta 3$, $V_\beta 5$, $V_\beta 6$, $V_\beta 7.3$, $V_\beta 7.4$, $V_\beta 9.1$, $V_\beta 12$, $V_\beta 14$, $V_\beta 15$, $V_\beta 17$ or $V_\beta 20$ chains.

10   A superantigen-encoding nucleic acid molecule of the present invention preferably encodes superantigens that includes, but is not limited to, staphylococcal enterotoxins, retroviral antigens, streptococcal antigens, mycoplasma antigens, mycobacterium antigens, viral antigens

15   (e.g., a superantigen from mouse mammary tumor virus, rabies virus or herpes virus) and endoparasitic antigens (e.g., protozoan or helminth antigens), more preferably staphylococcal enterotoxins, and even more preferably *Staphylococcal enterotoxin* A (SEA), *Staphylococcal*

20   *enterotoxin* B (SEB), *Staphylococcal enterotoxin* C_1 (SEC$_1$), *Staphylococcal enterotoxin* C_2 (SEC$_2$), *Staphylococcal enterotoxin* C_3 (SEC$_3$), *Staphylococcal enterotoxin* D (SED), *Staphylococcal enterotoxin* E (SEE) and Toxic Shock Syndrome Toxin (TSST).

25   A preferred nucleic acid molecule encoding a *Staphylococcal enterotoxin* of the present invention comprises a nucleic acid sequence represented by SEQ ID NO:1 (representing a full-length SEB gene), SEQ ID NO:3

(representing a full-length SEA gene) or SEQ ID NO:6
(representing a full-length TSST gene). A preferred
Staphylococcal enterotoxin protein of the present invention
comprises an amino acid sequence represented by SEQ ID NO:2

5   (representing a full-length SEB protein), SEQ ID NO:4
(representing a full-length SEA protein) or SEQ ID NO:7
(representing a full-length TSST protein).

In a preferred embodiment, a nucleic acid molecule of
the present invention encoding a superantigen comprises a

10   nucleic acid sequence spanning base pair 46 to at least
base pair 768 of SEQ ID NO:1, a nucleic acid sequence
spanning base pair 46 to about base pair 751 of SEQ ID NO:3
or SEQ ID NO:6.

Another embodiment of the present invention includes

15   a cytokine-encoding nucleic acid molecule that encodes a
full-length cytokine or a homologue of the cytokine
protein. As used herein, a homologue of a cytokine is a
protein having an amino acid sequence that is sufficiently
similar to a natural cytokine amino acid sequence so as to

20   have cytokine activity. In accordance with the present
invention, a cytokine includes a protein that is capable of
affecting the biological function of another cell. A
biological function affected by a cytokine can include, but
is not limited to, cell growth, cell differentiation or

25   cell death. Preferably, a cytokine of the present
invention is capable of binding to a specific receptor on
the surface of a cell, thereby affecting the biological
function of a cell.

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A cytokine-encoding nucleic acid molecule of the present invention encodes a cytokine that is capable of affecting the biological function of a cell, including, but not limited to, a lymphocyte, a muscle cell, a hematopoietic precursor cell, a mast cell, a natural killer cell, a macrophage, a monocyte, an epithelial cell, an endothelial cell, a dendritic cell, a mesenchymal cell, a Langerhans cell, cells found in granulomas and tumor cells of any cellular origin, and more preferably a mesenchymal cell, an epithelial cell, an endothelial cell, a muscle cell, a macrophage, a monocyte, a T cell and a dendritic cell.

A preferred cytokine nucleic acid molecule of the present invention encodes a hematopoietic growth factor, an interleukin, an interferon, an immunoglobulin superfamily molecule, a tumor necrosis factor family molecule and/or a chemokine (i.e., a protein that regulates the migration and activation of cells, particularly phagocytic cells). A more preferred cytokine nucleic acid molecule of the present invention encodes a granulocyte macrophage colony stimulating factor (GM-CSF), tumor necrosis factor α (TNF-α), macrophage colony stimulating factor (M-CSF), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-12 (IL-12), interleukin-15 (IL-15) and/or IGIF. An even more preferred cytokine nucleic acid molecule of the present invention encodes GM-CSF, IL-2, IL-12, IGIF and/or TNF-α, with GM-CSF being even more preferred.

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As will be apparent to one of skill in the art, the present invention is intended to apply to cytokines derived from all types of animals. A preferred animal from which to derive cytokines includes a mouse, a human, a cat and a

5   dog. A more preferred animal from which to derive cytokines includes a cat, a dog and a human. An even more preferred animal from which to derive cytokines is a human.

According to the present invention, a cytokine-encoding nucleic acid molecule of the present invention is

10 derived from the same species of animal as the animal to be treated. For example, a cytokine-encoding nucleic acid molecule derived from a canine (i.e., dog) nucleic acid molecule is used to treat a disease in a canine. Thus, a preferred cytokine-encoding nucleic acid molecule of the

15 present invention comprises a nucleic acid molecule encoding human GM-CSF, as described in the art. A human GM-CSF-encoding nucleic acid molecule of the present invention can be produced using methods standard PCR amplification methods with primers designed from the human

20 GM-CSF nucleic acid sequence disclosed in Nash (*Blood* 78:930, 1991). Such PCR products can be cloned into a PCR_3 expression vector using the methods generally described in Example 1.

Another embodiment of the present invention includes

25 a chemokine-encoding nucleic acid molecule that encodes a full-length chemokine or a homologue of the chemokine protein. As used herein, a homologue of a chemokine is a protein having an amino acid sequence that is sufficiently

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similar to a natural chemokine amino acid sequence so as to have chemokine activity. In accordance with the present invention, a chemokine includes a protein that is capable of attracting cells involved in an immune response

5   (immunologic cells), including phagocytic cells. For example, immunologic cells are recruited from the blood to a site at which the chemokine is located (e.g., a site of infection). Preferably, a chemokine of the present invention is capable of binding to a specific receptor on

10 the surface of a cell, thereby attracting the cell to a specific location.

A chemokine-encoding nucleic acid molecule of the present invention encodes a chemokine that is capable of attracting a cell to a site, including, but not limited to,

15 a dendritic cell, a neutrophil, a macrophage, a T lymphocyte and Langerhans cells, and more preferably a dendritic cell, a macrophage and a T lymphocyte.

A preferred chemokine-encoding nucleic acid molecule of the present invention encodes an α-chemokine or a β-

20 chemokine. A more preferred chemokine-encoding nucleic acid molecule of the present invention encodes a C5a, interleukin-8 (IL-8), monocyte chemotactic protein 1α (MIP1α), monocyte chemotactic protein 1β (MIP1β), monocyte chemoattractant protein 1 (MCP-1), monocyte chemoattractant

25 protein 3 (MCP-3), platelet activating factor (PAFR), N-Formyl-methionyl-leucyl-[^{3H}]phenylalanine (FMLPR), leukotriene B$_4$ (LTB$_4$R), gastrin releasing peptide (GRP), RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2,

NAP-2 and/or MGSA/gro. An even more preferred chemokine-encoding nucleic acid molecule of the present invention encodes IL-8, MIP1α, MIP1β, MCP-1, MCP-3, RANTES and/or NAP-2, with IL-8, Rantes, MIP1α and/or MIP1β being even

5   more preferred.

As will be apparent to one of skill in the art, the present invention is intended to apply to chemokines derived from all types of animals. Preferred animals from which to derive chemokines includes mice, humans, dogs,

10   cats, cattle and horses. More preferred animals from which to derive chemokines includes dogs, cats, humans and cattle. Even more preferred animals from which to derive chemokines are humans.

According to the present invention, a chemokine-

15   encoding nucleic acid molecule of the present invention is derived from the same species of animal as the animal to be treated. For example, a chemokine-encoding nucleic acid molecule derived from a canine (i.e., dog) nucleic acid molecule is used to treat a disease in a canine. Thus, a

20   preferred chemokine-encoding nucleic acid molecule of the present invention comprises a nucleic acid molecule encoding a dog, cat, human, bovine and/or equine chemokine. Preferred nucleic acid molecules of the present invention encode IL-8, Rantes, MIP1α and/or MIP1β, as described in

25   the art. For example, a human MIP1α-encoding nucleic acid molecule of the present invention can be produced using standard PCR amplification methods with primers designed from the human MIP1α-encoding nucleic acid sequence

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disclosed in the art. Such PCR products can be cloned into a PCR₃ expression vector using the methods generally described in Example 1.

The present invention includes a nucleic acid molecule
5   of the present invention operatively linked to one or more transcription control sequences to form a recombinant molecule. The phrase "operatively linked" refers to linking a nucleic acid molecule to a transcription control sequence in a manner such that the molecule is able to be
10   expressed when transfected (i.e., transformed, transduced or transfected) into a host cell. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which
15   control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of
20   such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in animal, bacteria, helminth, insect cells, and preferably in animal cells. More preferred transcription control sequences include, but
25   are not limited to, simian virus 40 (SV-40), β-actin, retroviral long terminal repeat (LTR), Rous sarcoma virus (RSV), cytomegalovirus (CMV), *tac, lac, trp, trc*, oxy-pro, omp/lpp, rrnB, bacteriophage lambda (λ) (such as λp_L and λp_R

and fusions that include such promoters), bacteriophage T7,
T7*lac*, bacteriophage T3, bacteriophage SP6, bacteriophage
SP01, metallothionein, alpha mating factor, *Pichia* alcohol
oxidase, alphavirus subgenomic promoters (such as Sindbis
5   virus subgenomic promoters), baculovirus, *Heliothis zea*
insect virus, vaccinia virus and other poxviruses,
herpesvirus, and adenovirus transcription control
sequences, as well as other sequences capable of
controlling gene expression in eukaryotic cells.
10 Additional suitable transcription control sequences include
tissue-specific promoters and enhancers (e.g., tumor cell-
specific enhancers and promoters), and inducible promoters
(e.g., tetracycline). Transcription control sequences of
the present invention can also include naturally occurring
15 transcription control sequences naturally associated with
a gene encoding a superantigen, a cytokine or a chemokine
of the present invention.

        Recombinant molecules of the present invention, which
can be either DNA or RNA, can also contain additional
20 regulatory sequences, such as translation regulatory
sequences, origins of replication, and other regulatory
sequences that are compatible with the recombinant cell.
In one embodiment, a recombinant molecule of the present
invention also contains secretory signals (i.e., signal
25 segment nucleic acid sequences) to enable an expressed
superantigen, cytokine or a chemokine protein to be
secreted from the cell that produces the protein. Suitable
signal segments include: (1) a bacterial signal segment, in

particular a superantigen signal segment; (2) a cytokine signal segment; (3) a chemokine signal segment; (4) or any heterologous signal segment capable of directing the secretion of a superantigen, cytokine and/or chemokine

5   protein of the present invention. Preferred signal segments include, but are not limited to, signal segments associated with SEB, SEA, TSST, GM-CSF, M-CSF, TNFα, IL-1, IL-2, IL-4, IL-6, IL-12, IL-15, C5a, IGIF, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB₄R, GRP, RANTES,

10 eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and/or MGSA/gro protein.

Preferred recombinant molecules of the present invention include a recombinant molecule containing a nucleic acid molecule encoding a superantigen, a

15 recombinant molecule containing a nucleic acid molecule encoding a cytokine, a recombinant molecule containing a nucleic acid molecule encoding a chemokine, a recombinant molecule containing a nucleic acid molecule encoding a superantigen and a nucleic acid molecule encoding a

20 cytokine to form a chimeric recombinant molecule, or a recombinant molecule containing a nucleic acid molecule encoding a superantigen and a nucleic acid molecule encoding a chemokine to form a chimeric recombinant molecule. The nucleic acid molecules contained in such

25 recombinant chimeric molecules are operatively linked to one or more transcription control sequences, in which each nucleic acid molecule contained in a chimeric recombinant molecule can be expressed using the same or different

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regulatory control sequences.   Preferred recombinant
molecules of the present invention comprise a nucleic acid
sequence represented by SEQ ID NO:1, SEQ ID NO:3, SEQ ID
NO:5, or combinations thereof.   Particularly preferred

5   recombinant molecules include PCR$_3$-SEB, PCR$_3$-SEA, PCR$_3$-SEB.S,
PCR$_3$-SEA.S, PCR$_3$-TSST and PCR$_3$-GM$_3$, the production of which
is disclosed herein. Other preferred nucleic acid sequences
include Rantes nucleic acid sequence (SEQ ID. NO:13), MIP1α
nucleic acid sequence (see Davatelis et al., *J. Exp. Med.*

10   167:1939-1944, 1988) and MIP1β nucleic acid sequence (see
Sherry et al., *J. Exp. Med.* 168:2251-2259, 1988).

According to the present invention, a recombinant
molecule can be dicistronic. A cistron refers to a unit of
DNA that is capable of encoding an amino acid sequence

15   having a naturally-occurring biological function.   A
dicistronic plasmid refers to a plasmid containing 2
cistrons.   Preferably, a dicistronic recombinant molecule
of the present invention comprises an internal ribosome
entry site (IRES) element to which eukaryotic ribosomes can

20   bind (see, for example, Jang et al., *J. Virol.* 62:2636-
2643, 1988; Pelletier et al. *Nature* 334:320-325, 1988;
Jackson, *Nature* 353:14-15, 1991; Macejek et al., *Nature*
353:90-94, 1991; Oh et al., *Genes & Develop.* 6:1643-1653,
1992; Molla et al., *Nature* 356:255-257, 1992; and Kozak,

25   *Crit. Rev. Biochem. Molec. Biol.* 27(4,5):385-402, 1992).

In one embodiment, a dicistronic recombinant molecule
of the present invention comprises a eukaryotic promoter,
operatively linked to a superantigen-encoding nucleic acid

molecule of the present invention and a cytokine-encoding nucleic acid molecule of the present invention separated by an IRES nucleic acid sequence, or a superantigen-encoding nucleic acid molecule of the present invention and

5   chemokine-encoding nucleic acid molecule of the present invention separated by an IRES nucleic acid sequence.

In another embodiment, a dicistronic recombinant molecule of the present invention comprises a eukaryotic promoter, operatively linked to a first superantigen-

10   encoding nucleic acid molecule of the present invention and a second superantigen-encoding nucleic acid molecule of the present invention separated by an IRES nucleic acid sequence.   Preferably, a first superantigen-encoding nucleic acid molecule encodes a different superantigen than

15   a second superantigen-encoding nucleic acid molecule.

One or more recombinant molecules of the present invention can be used to produce an encoded product (i.e., a superantigen protein, a cytokine and a chemokine protein) of the present invention.   In one embodiment, an encoded

20   product of the present invention is produced by expressing a nucleic acid molecule of the present invention in a cell under conditions effective to produce the protein.   A preferred method to produce an encoded protein is by transforming (i.e., introducing a recombinant molecule into

25   a cell in such a manner that the recombinant molecule is expressed by the cell) a host cell with one or more recombinant molecules of the present invention to form a recombinant cell. Suitable host cells to transform include

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any cell into which a recombinant molecule can be
introduced. Host cells can be either untransformed cells
or cells that are already transformed with at least one
nucleic acid molecule. Host cells of the present invention
5   can be any cell capable of producing a superantigen, a
cytokine and/or a chemokine of the present invention,
including bacterial, fungal, animal parasite, insect and
animal cells. A preferred host cell includes a mammalian
and a bird cell. A more preferred host cell includes
10 mammalian lymphocytes, muscle cells, hematopoietic
precursor cells, mast cells, natural killer cells,
macrophages, monocytes, epithelial cells, endothelial
cells, dendritic cells, mesenchymal cells, Langerhans
cells, cells found in granulomas and tumor cells of any
15 cellular origin. An even more preferred host cell of the
present invention includes mammalian mesenchymal cells,
epithelial cells, endothelial cells, macrophages,
monocytes, muscle cells, T cells and dendritic cells.

According to the present invention, a recombinant
20 molecule can be introduced into a host cell *in vivo* (i.e.,
in an animal) or *in vitro* (i.e., outside of an animal, such
as in tissue culture). Introduction of a nucleic acid
molecule into a host cell can be accomplished by any method
by which a nucleic acid molecule can be inserted into the
25 cell. Transformation techniques include, but are not
limited to, transfection, electroporation, microinjection,
lipofection, adsorption, and protoplast fusion. Preferred
methods to introduce a recombinant molecule into host cells

in vivo include lipofection and adsorption (discussed in detail below).

A recombinant cell of the present invention comprises a cell into which a nucleic acid molecule that encodes a

5   superantigen, a cytokine and/or a chemokine has been introduced. In one embodiment, a recombinant cell of the present invention is transformed with a nucleic acid molecule that includes at least a portion of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST, or combinations

10   thereof. Particularly preferred recombinant cells include cells transformed with PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S or PCR_3-TSST, with PCR_3-SEB.S, PCR_3-SEA.S or PCR_3-TSST being even more preferred.

In another embodiment, a recombinant cell of the

15   present invention is transformed with a nucleic acid molecule that includes at least a portion of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST or combinations thereof, and PCR_3-GM_3. Particularly preferred stimulatory recombinant cells include cells transformed with PCR_3-SEA

20   and PCR_3-GM_3, PCR_3-SEA.S and PCR_3-GM_3, PCR_3-SEB and PCR_3-GM_3, PCR_3-SEB.S and PCR_3-GM_3, or PCR_3-TSST and PCR_3-GM_3. Even more preferred stimulatory recombinant cells include cells transformed with PCR_3-SEB.S and PCR_3-GM_3, or PCR_3-SEA.S and PCR_3-GM_3, and PCR_3-TSST and PCR_3-GM_3.

25   Recombinant DNA technologies can be used to improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency

with which those nucleic acid molecules are transcribed,
the efficiency with which the resultant transcripts are
translated, and the efficiency of post-translational
modifications. Recombinant techniques useful for increasing
5   the expression of nucleic acid molecules of the present
invention include, but are not limited to, operatively
linking nucleic acid molecules to high-copy number
plasmids, integration of the nucleic acid molecules into
one or more host cell chromosomes, addition of vector
10 stability sequences to plasmids, substitutions or
modifications of transcription control signals (e.g.,
promoters, operators, enhancers), substitutions or
modifications of translational control signals (e.g.,
ribosome binding sites, Shine-Dalgarno sequences),
15 modification of nucleic acid molecules of the present
invention to correspond to the codon usage of the host
cell, and deletion of sequences that destabilize
transcripts. The activity of an expressed recombinant
protein of the present invention may be improved by
20 fragmenting, modifying, or derivatizing nucleic acid
molecules encoding such a protein.

Further embodiments of compositions of the present
invention can also include a compound capable of inhibiting
the downregulation of T cell activity. In particular, such
25 a compound can include an inhibitor of CTLA-4. An
inhibitor of CTLA-4 includes any compound capable of
inhibiting the activity of CTLA-4 and/or inhibiting the
binding of CTLA-4 to its natural ligand (e.g., B7).

Preferably, an inhibitor of CTLA-4 includes, but is not limited to a ligand of CTLA-4 or an analog (i.e., antagonist) of CTLA-4. Preferred ligands of CTLA-4 include: an antibody that specifically binds to CTLA-4 in

5   such a manner that CTLA-4 activity is inhibited; at least a portion of a B7 molecule, in particular a B7 fusion protein; or a synthetic oligonucleotide that binds CTLA-4 protein. A preferred analog of CTLA-4 includes a molecule capable of binding to B7 in such a manner that B7 signal

10   transduction is not activated and CTLA-4 binding to the B7 molecule is inhibited. It is within the scope of the invention that a CTLA-4 inhibitor can comprise a nucleic acid molecule, a protein or a synthetic chemical molecule when combined in a composition of the present invention.

15   In another embodiment of the present invention, a therapeutic composition further comprises a pharmaceutically acceptable carrier. As used herein, a "carrier" refers to any substance suitable as a vehicle for delivering a nucleic acid molecule of the present invention

20   to a suitable *in vivo* or *in vitro* site. As such, carriers can act as a pharmaceutically acceptable excipient of a therapeutic composition containing a nucleic acid molecule of the present invention. Preferred carriers are capable of maintaining a nucleic acid molecule of the present

25   invention in a form that, upon arrival of the nucleic acid molecule to a cell, the nucleic acid molecule is capable of entering the cell and being expressed by the cell. Carriers of the present invention include: (1) excipients

or formularies that transport, but do not specifically target a nucleic acid molecule to a cell (referred to herein as non-targeting carriers); and (2) excipients or formularies that deliver a nucleic acid molecule to a

5   specific site in an animal or a specific cell (i.e., targeting carriers). Examples of non-targeting carriers include, but are not limited to water, phosphate buffered saline, Ringer's solution, dextrose solution, serum-containing solutions, Hank's solution, other aqueous

10   physiologically balanced solutions, oils, esters and glycols. Aqueous carriers can contain suitable auxiliary substances required to approximate the physiological conditions of the recipient, for example, by enhancing chemical stability and isotonicity.

15   Suitable auxiliary substances include, for example, sodium acetate, sodium chloride, sodium lactate, potassium chloride, calcium chloride, and other substances used to produce phosphate buffer, Tris buffer, and bicarbonate buffer. Auxiliary substances can also include

20   preservatives, such as thimerosal, m- and o-cresol, formalin and benzol alcohol. Preferred auxiliary substances for aerosol delivery include surfactant substances non-toxic to an animal, for example, esters or partial esters of fatty acids containing from about six to

25   about twenty-two carbon atoms. Examples of esters include, caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric, and oleic acids. Other carriers can include metal particles (e.g., gold particles) for use

with, for example, a biolistic gun through the skin. Therapeutic compositions of the present invention can be sterilized by conventional methods and/or lyophilized.

Targeting carriers are herein referred to as "delivery 5 vehicles." Delivery vehicles of the present invention are capable of delivering a therapeutic composition of the present invention to a target site in an animal. A "target site" refers to a site in an animal to which one desires to deliver a therapeutic composition. For example, a target 10 site can be a malignant tumor cell, a non-malignant tumor cell, a lymph node or a lesion caused by an infectious agent, or an area around such cell, tumor or lesion, which is targeted by direct injection or delivery using liposomes or other delivery vehicles. Examples of delivery vehicles 15 include, but are not limited to, artificial and natural lipid-containing delivery vehicles. Natural lipid-containing delivery vehicles include cells and cellular membranes. Artificial lipid-containing delivery vehicles include liposomes and micelles. A delivery vehicle of the 20 present invention can be modified to target to a particular site in an animal, thereby targeting and making use of a nucleic acid molecule of the present invention at that site. Suitable modifications include manipulating the chemical formula of the lipid portion of the delivery 25 vehicle and/or introducing into the vehicle a compound capable of specifically targeting a delivery vehicle to a preferred site, for example, a preferred cell type. Specifically targeting refers to causing a delivery vehicle

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to bind to a particular cell by the interaction of the compound in the vehicle to a molecule on the surface of the cell. Suitable targeting compounds include ligands capable of selectively (i.e., specifically) binding another

5 molecule at a particular site. Examples of such ligands include antibodies, antigens, receptors and receptor ligands. For example, an antibody specific for an antigen found on the surface of a cancer cell can be introduced to the outer surface of a liposome delivery vehicle so as to

10 target the delivery vehicle to the cancer cell. Tumor cell ligands include ligands capable of binding to a molecule on the surface of a tumor cell. Manipulating the chemical formula of the lipid portion of the delivery vehicle can modulate the extracellular or intracellular targeting of

15 the delivery vehicle. For example, a chemical can be added to the lipid formula of a liposome that alters the charge of the lipid bilayer of the liposome so that the liposome fuses with particular cells having particular charge characteristics.

20   A preferred delivery vehicle of the present invention is a liposome. A liposome is capable of remaining stable in an animal for a sufficient amount of time to deliver a nucleic acid molecule of the present invention to a preferred site in the animal. A liposome of the present

25 invention is preferably stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour and even more preferably for at least about 24 hours.

A liposome of the present invention comprises a lipid composition that is capable of targeting a nucleic acid molecule of the present invention to a particular, or selected, site in an animal. Preferably, the lipid

5 composition of the liposome is capable of targeting to any organ of an animal, more preferably to the lung, liver, spleen, heart brain, lymph nodes and skin of an animal, and even more preferably to the lung of an animal.

A liposome of the present invention comprises a lipid

10 composition that is capable of fusing with the plasma membrane of the targeted cell to deliver a nucleic acid molecule into a cell. Preferably, the transfection efficiency of a liposome of the present invention is at least about 0.5 microgram (μg) of DNA per 16 nanomole

15 (nmol) of liposome delivered to about 10^6 cells, more preferably at least about 1.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells, and even more preferably at least about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells.

20 A preferred liposome of the present invention is between about 100 and about 500 nanometers (nm), more preferably between about 150 and about 450 nm and even more preferably between about 200 and about 400 nm in diameter.

Suitable liposomes for use with the present invention

25 include any liposome. Preferred liposomes of the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes comprise liposomes

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having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Even more preferred liposomes include liposomes produced according to the method described in Example 2.

5   In one embodiment, a liposome of the present invention comprises a compound capable of targeting the liposome to a tumor cell. Such a liposome preferably includes a tumor cell ligand exposed on the outer surface of the liposome.

Complexing a liposome with a nucleic acid molecule of 10   the present invention can be achieved using methods standard in the art (see, for example, methods described in Example 2). A suitable concentration of a nucleic acid molecule of the present invention to add to a liposome includes a concentration effective for delivering a 15   sufficient amount of nucleic acid molecule to a cell such that the cell can produce sufficient superantigen and/or cytokine protein to regulate effector cell immunity in a desired manner. Preferably, nucleic acid molecules are combined with liposomes at a ratio of from about 0.1 μg to 20   about 10 μg of nucleic acid molecule of the present invention per about 8 nmol liposomes, more preferably from about 0.5 μg to about 5 μg of nucleic acid molecule per about 8 nmol liposomes, and even more preferably about 1.0 μg of nucleic acid molecule per about 8 nmol liposomes.

25   Another preferred delivery vehicle comprises a recombinant virus particle vaccine. A recombinant virus particle vaccine of the present invention includes a therapeutic composition of the present invention, in which

the recombinant molecules contained in the composition are packaged in a viral coat that allows entrance of DNA into a cell so that the DNA is expressed in the cell. A number of recombinant virus particles can be used, including, but not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, arena virus and retroviruses.

Another preferred delivery vehicle comprises a recombinant cell vaccine. Preferred recombinant cell vaccines of the present invention include tumor vaccines, in which allogeneic (i.e., cells derived from a source other than a patient, but that are histiotype compatible with the patient) or autologous (i.e., cells isolated from a patient) tumor cells are transfected with recombinant molecules contained in a therapeutic composition, irradiated and administered to a patient by, for example, intradermal, intravenous or subcutaneous injection. Therapeutic compositions to be administered by tumor cell vaccine, include recombinant molecules of the present invention without carrier. Tumor cell vaccine treatment is useful for the treatment of both tumor and metastatic cancer. Use of a tumor vaccine of the present invention is particular useful for treating metastatic cancer, including preventing metastatic disease, as well as, curing existing metastatic disease. Methods for developing and administering include those standard in the art (see for example, Dranoff et al., *Proc. Natl. Acad. Sci. USA* 90:3539-3543, 1993, which is incorporated herein by reference in its entirety).

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A therapeutic composition of the present invention is useful for the treatment of a variety of diseases, including, but not limited to, cancer, autoimmune disease, infectious diseases, and other diseases that can be

5   alleviated by either stimulating or suppressing T cell activity. As used herein, the term "treatment" refers to protecting an animal from a disease or alleviating a disease in an animal. A therapeutic composition of the present invention is advantageous for the treatment of

10   cancer in that the composition overcomes the mechanisms by which cancer cells avoid immune elimination (i.e., by which cancer cells avoid the immune response effected by the animal in response to the disease). Cancer cells can avoid immune elimination by, for example, being only slightly

15   immunogenic, modulating cell surface antigens and inducing immune suppression. Suitable therapeutic compositions for use in the treatment of cancer comprises a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a

20   cytokine-encoding recombinant molecule and/or a chemokine recombinant molecule of the present invention. Preferred therapeutic compositions for use in the treatment of cancer comprises a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant

25   molecule with a cytokine-encoding recombinant molecule and/or a chemokine recombinant molecule of the present invention combined (separately or together) with a delivery vehicle, preferably a liposome, such as disclosed herein.

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A therapeutic composition of the present invention, upon entering targeted cells, leads to the production of superantigen, cytokine and/or chemokine protein that activate cytotoxic T cells, natural killer cells, T helper

5   cells and macrophages. Such cellular activation overcomes the otherwise relative lack of immune response to cancer cells, leading to the destruction of such cells.

A therapeutic composition of the present invention is useful for the treatment of cancers, both tumors and

10   metastatic forms of cancer. Treatment with the therapeutic composition overcomes the disadvantages of traditional treatments for metastatic cancers. For example, compositions of the present invention can target dispersed metastatic cancer cells that cannot be treated using

15   surgical methods. In addition, administration of such compositions do not result in the harmful side effects caused by chemotherapy and radiation therapy.

A therapeutic composition of the present invention is preferably used to treat cancers, including, but not

20   limited to, melanomas, squamous cell carcinoma, breast cancers, head and neck carcinomas, thyroid carcinomas, soft tissue sarcomas, bone sarcomas, testicular cancers, prostatic cancers, ovarian cancers, bladder cancers, skin cancers, brain cancers, angiosarcomas, hemangiosarcomas,

25   mast cell tumors, primary hepatic cancers, lung cancers, pancreatic cancers, gastrointestinal cancers, renal cell carcinomas, hematopoietic neoplasias, leukemias and lymphomas. Particularly preferred cancers to treat with a

therapeutic composition of the present invention, include
melanomas, lung cancers, thyroid carcinomas, breast
cancers, renal cell carcinomas, squamous cell carcinomas,
brain tumors and skin cancers. A therapeutic composition

5   of the present invention is useful for treating tumors that
can form in such cancers, including malignant and benign
tumors.

A therapeutic composition of the present invention is
also advantageous for the treatment of infectious diseases

10   as a long term, targeted therapy for primary lesions (e.g.,
granulomas) resulting from the propagation of a pathogen.
As used herein, the term "lesion" refers to a lesion formed
by infection of an animal with a pathogen. Preferred
therapeutic compositions for use in the treatment of an

15   infectious disease comprise a superantigen-encoding
recombinant molecule; or a combination of a superantigen-
encoding recombinant molecule, with a cytokine-encoding
recombinant molecule and/or a chemokine recombinant
molecule of the present invention.   More preferred

20   therapeutic compositions for use in the treatment of
infectious disease comprise a superantigen-encoding
recombinant molecule; or a combination of superantigen-
encoding recombinant molecule, with a cytokine-encoding
recombinant molecule and/or a chemokine recombinant

25   molecule of the present invention combined with a delivery
vehicle, preferably a liposome of the present invention.
Similar to the mechanism described for the treatment of
cancer, treatment of infectious diseases with superantigen,

cytokine and/or chemokine can result in increased T cell, natural killer cell, and macrophage cell activity that overcome the relative lack of immune response to a lesion formed by a pathogen.

5   A therapeutic composition of the present invention is particularly useful for the treatment of infectious diseases caused by pathogens, including, but not limited to, intracellular bacteria (i.e., a bacteria that resides in a host cell), internal parasites, pathogenic fungi and

10 endoparasites. Particularly preferred infectious diseases to treat with a therapeutic composition of the present invention include tuberculosis, leprosy, aspergillosis, coccidioidomycosis, cryptococcoses, leishmaniasis and toxoplasmosis.

15 In order to treat an animal with disease, a therapeutic composition of the present invention is administered to the animal in an effective manner such that the composition is capable of treating that animal from disease. For example, a recombinant molecule, when

20 administered to an animal in an effective manner, is able to stimulate effector cell immunity in a manner that is sufficient to alleviate the disease afflicting the animal. According to the present invention, treatment of a disease refers to alleviating a disease and/or preventing the

25 development of a secondary disease resulting from the occurrence of a primary disease.

An effective administration protocol (i.e., administering a therapeutic composition in an effective

manner) comprises suitable dose parameters and modes oʌ administration that result in treatment of a disease. Effective dose parameters and modes of administration can be determined using methods standard in the art for a

5   particular disease. Such methods include, for example, determination of survival rates, side effects (i.e., toxicity) and progression or regression of disease. In particular, the effectiveness of dose parameters and modes of administration of a therapeutic composition of the

10   present invention when treating cancer can be determined by assessing response rates. Such response rates refer to the percentage of treated patients in a population of patients that respond with either partial or complete remission. Remission can be determined by, for example, measuring

15   tumor size or microscopic examination for the presence of cancer cells in a tissue sample.

In accordance with the present invention, a suitable single dose size is a dose that is capable of treating an animal with disease when administered one or more times

20   over a suitable time period. Doses can vary depending upon the disease being treated. In the treatment of cancer, a suitable single dose can be dependent upon whether the cancer being treated is a primary tumor or a metastatic form of cancer. Doses of a therapeutic composition of the

25   present invention suitable for use with direct injection techniques can be used by one of skill in the art to determine appropriate single dose sizes for systemic administration based on the size of an animal. A suitable

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single dose of a therapeutic composition to treat a tumor is a sufficient amount of a superantigen-encoding recombinant molecule; or a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant

5   molecule and/or a chemokine recombinant molecule to reduce, and preferably eliminate, the tumor following transfection of the recombinant molecules into cells at or near the tumor site. A preferred single dose of the superantigen-encoding recombinant molecule is an amount that, when

10   transfected into a target cell population, leads to the production of from about 250 femtograms (fg) to about 1 μg, preferably from about 500 fg to about 500 picogram (pg), and more preferably from about 1 pg to about 100 pg of superantigen per transfected cell. A preferred single dose

15   of a cytokine-encoding recombinant molecule is an amount that, when transfected into a target cell population, leads to the production of from about 10 pg to about 1 μg, preferably from about 100 pg to about 750 pg, and more preferably about 500 pg of cytokine per transfectant. A

20   preferred single dose of a chemokine-encoding recombinant molecule is an amount that, when transfected into a target cell population, leads to the production of from about 1 fg to about 1 μg, preferably from about 1 pg to about 10 ng, and more preferably from about 1 pg to about 1 ng chemokine

25   per transfectant.

A suitable single dose of a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding

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recombinant molecule and/or a chemokine-encoding recombinant molecule in a non-targeting carrier to administer to an animal to treat a tumor, is an amount capable of reducing, and preferably eliminating, the tumor

5   following transfection of the recombinant molecules into cells at or near the tumor site. A preferred single dose of a therapeutic composition to treat a tumor is from about 100 μg to about 2 milligrams (mg) of total recombinant molecules, more preferably from about 150 μg to about 1 mg

10   of total recombinant molecules, and even more preferably from about 200 μg to about 800 μg of total recombinant molecules. A preferred single dose of a superantigen-encoding recombinant molecule complexed with liposomes, is from about 100 μg of total DNA per 800 nmol of liposome to

15   about 2 mg of total recombinant molecules per 16 micromole (μmol) of liposome, more preferably from about 150 μg per 1.2 μmol of liposome to about 1 mg of total recombinant molecules per 8 μmol of liposome, and even more preferably from about 200 μg per 2 μmol of liposome to about 400 μg of

20   total recombinant molecules per 3.2 μmol of liposome.

A preferred single dose of a cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule in a non-targeting carrier to administer to an animal to treat a tumor, is from about 100 μg to about 2 mg

25   of total recombinant molecules, more preferably from about 150 μg to about 1 mg of total recombinant molecules, and even more preferably from about 200 μg to about 400 μg of total recombinant molecules. A preferred single dose of a

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cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule complexed with liposomes to administer to an animal to treat a tumor, is from about 100 μg of total recombinant molecules per 800 nmol of liposome

5   to about 2 mg of total recombinant molecules per 16 μmol of liposome, more preferably from about 150 μg per 1.2 μmol of liposome to about 1 mg of total recombinant molecules per 8 μmol of liposome, and even more preferably from about 200 μg per 2 μmol of liposome to about 400 μg of total

10   recombinant molecules per 6.4 μmol of liposome.

A preferred single dose of a superantigen-encoding recombinant molecule in a non-targeting carrier to administer to an animal treat a metastatic cancer, is from about 100 μg to about 4 mg of total recombinant molecules,

15   more preferably from about 150 μg to about 3 mg of total recombinant molecules, and even more preferably from about 200 μg to about 2 mg of total recombinant molecules. A preferred single dose of a superantigen-encoding recombinant molecule complexed with liposomes to administer

20   to an animal to treat a metastatic cancer, is from about 100 μg of total recombinant molecules per 800 nmol of liposome to about 4 mg of total recombinant molecules per 32 μmol of liposome, more preferably from about 200 μg per 1.6 μm of liposome to about 3 mg of total recombinant

25   molecules per 24 μmol of liposome, and even more preferably from about 400 μg per 3.2 μmol of liposome to about 2 mg of total recombinant molecules per 16 μmol of liposome.

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A preferred single dose of a cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule in a non-targeting carrier to administer to an animal to treat a metastatic cancer, is from about 100 µg

5   to about 4.0 mg of total recombinant molecules, more preferably from about 150 µg to about 3 mg of total recombinant molecules, and even more preferably from about 200 µg to about 2 mg of total recombinant molecules. A preferred single dose of a cytokine-encoding recombinant

10   molecule or a chemokine-encoding recombinant molecule complexed with liposomes to administer to an animal to treat a metastatic cancer, is from about 100 µg of total recombinant molecules per 800 nmol of liposome to about 4.0 mg of total recombinant molecules per 32 µmol of liposome,

15   more preferably from about 200 µg per 1.6 µmol of liposome to about 3 mg of total recombinant molecules per 24 µmol of liposome, and even more preferably from about 400 µg per 3.2 µmol of liposome to about 2 µg of total recombinant molecules per 16 µmol of liposome.

20   According to the present invention, a single dose of a therapeutic composition useful to treat a lesion, comprising a superantigen-encoding recombinant molecule in a non-targeting carrier or liposomes, respectively, and a cytokine-encoding recombinant molecule in a non-targeting

25   carrier or liposomes, respectively, is substantially similar to those doses used to treat a tumor (as described in detail above).

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The number of doses administered to an animal is dependent upon the extent of the disease and the response of an individual patient to the treatment. For example, a large tumor may require more doses than a smaller tumor.

5   In some cases, however, a patient having a large tumor may require fewer doses than a patient with a smaller tumor, if the patient with the large tumor responds more favorably to the therapeutic composition than the patient with the smaller tumor. Thus, it is within the scope of the present

10   invention that a suitable number of doses includes any number required to cause regression of a disease. A preferred protocol is monthly administrations of single doses (as described above) for up to about 1 year. A preferred number of doses of a therapeutic composition

15   comprising a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule in a non-targeting carrier or complexed with liposomes in order to

20   treat a tumor is from about 1 to about 10 administrations per patient, preferably from about 2 to about 8 administrations per patient, and even more preferably from about 3 to about 5 administrations per patient. Preferably, such administrations are given once every 2

25   weeks until signs of remission appear, then once a month until the disease is gone.

A preferred number of doses of a therapeutic composition comprising a superantigen-encoding recombinant

molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule in a non-targeting carrier or complexed with liposomes in

5   order to treat a metastatic cancer, is from about 2 to about 10 administrations patient, more preferably from about 3 to about 8 administrations per patient, and even more preferably from about 3 to about 7 administrations per patient. Preferably, such administrations are given once

10   every 2 weeks until signs of remission appear, then once a month until the disease is gone.

According to the present invention, the number of doses of a therapeutic composition to treat a lesion comprising a superantigen-encoding recombinant molecule; or

15   a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule, in a non-targeting carrier or liposomes, respectively, is substantially similar to those number of doses used to

20   treat a tumor (as described in detail above).

A therapeutic composition is administered to an animal in a fashion to enable expression of an introduced recombinant molecule of the present invention into a curative protein in the animal to be treated for disease.

25   A therapeutic composition can be administered to an animal in a variety of methods including, but not limited to, local administration of the composition into a site in an animal. Examples of such sites include lymph nodes, a site

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that contains abnormal cells or pathogens to be destroyed
(e.g., injection locally within the area of a tumor or a
lesion); and systemic administration.

Therapeutic compositions to be delivered by local
administration include: (a) recombinant molecules of the
present invention in a non-targeting carrier (e.g., as
"naked" DNA molecules, such as is taught, for example in
Wolff et al., 1990, *Science 247*, 1465-1468); and (b)
recombinant molecules of the present invention complexed to
a delivery vehicle of the present invention. Suitable
delivery vehicles for local administration comprise
liposomes. Delivery vehicles for local administration can
further comprise ligands for targeting the vehicle to a
particular site (as described in detail herein).

A preferred method of local administration is by
direct injection. Direct injection techniques are
particularly useful for the treatment of disease by, for
example, injecting the composition into a mass formed by
abnormal cells, a lymph node or a granuloma mass induced by
pathogens. Preferably, a recombinant molecule of the
present invention complexed with a delivery vehicle is
administered by direct injection into or locally within the
area of a tumor mass, a lymph node, a granuloma mass or a
cancer cell. Administration of a composition locally
within an area of a mass or a cell refers to injecting the
composition centimeters and preferably, millimeters within
the mass or the cell. A preferred tumor mass to inject
includes discrete inner body and cutaneous solid tumors.

A preferred inner body tumor to inject includes a discrete
solid tumor that forms in the brain, breast, liver, kidney,
colon, prostate, testicular, ovary, spleen and/or lymph
node. A preferred cutaneous tumor to inject includes a
5   discrete solid melanoma.

A preferred lymph node to inject includes a draining
lymph node that "drains" a site containing abnormal cells
or pathogens. As used herein, the term "draining lymph
node" refers to a lymph node that is located downstream of
10   a site containing abnormal cells or pathogens is based on
the direction of the lymphatic flow of an animal (see
general discussion in Hole, *Human Anatomy and Physiology*,
Edward G. Jaffe, ed., Wm. C Brown Publishers, Dubuque, IA;
and G.C. Christiansen et al., *Anatomy of the Dog*, W.B.
15   Saunders Publishers, Philadelphia, PN, 1979; both of which
are incorporated herein by this reference). A preferred
draining lymph node to inject comprises the draining lymph
node most proximal to a site containing abnormal cells or
pathogens. Thus, a skilled artisan can choose the site of
20   lymph node injection based upon the location of the site
containing abnormal cells or pathogens. Examples of lymph
nodes to injection include: the mandibular lymph node if a
tumor is located in the oral cavity; and the superficial
cervical lymph node of a tumor is located in the front leg
25   region. Effector cells travel from a site containing
abnormal cells or pathogens. Injection of a therapeutic
composition of the present invention into a lymph node can
result in expression of a superantigen, a cytokine and/or

a chemokine by an effector cell from the lymph node or that has drained into the lymph node. Such expression can result in the activation of T lymphocytes, which can travel back to the site containing abnormal cells or pathogens to

5   enhance the immune response at the site.

Another method of local administration is to contact a therapeutic composition of the present invention in or around a surgical wound. For example, a patient can undergo surgery to remove a tumor. Upon removal of the tumor, the

10   therapeutic composition can be coated on the surface of tissue inside the wound or the composition can be injected into areas of tissue inside the wound. Such local administration is useful for treating cancer cells not excised by the surgical procedure, as well as, preventing

15   recurrence of the primary tumor or development of a secondary tumor in the area of the surgery.

In one embodiment, a therapeutic composition of the present invention can be introduced to a tumor cell *in vivo*. In another embodiment, a therapeutic composition of

20   the present invention can be introduced to a non-tumor cell *in vivo* or *in vitro*. Methods to introduce a therapeutic composition *in vivo* are disclosed herein. Methods to introduce a therapeutic composition *in vitro* include methods standard in the art, such as culturing cells in the

25   presence of a therapeutic composition for a sufficient amount of time to enable a nucleic acid molecule of the present invention to pass through the plasma membrane in a cell and subsequently to be expressed in the cell.

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Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site, preferably ligands for targeting the vehicle to a site of a cancer or a lesion (depending upon the disease being treated). For cancer treatment, ligands capable of selectively binding to a cancer cell or to a cell within the area of a cancer cell are preferred. Systemic administration is useful for the treatment of both tumor and metastatic cancer and systemic infectious diseases. Systemic administration is particularly useful for the treatment of metastatic forms of cancer, in which the cancer cells are dispersed (i.e., not localized within a single tumor mass). Systemic administration is particularly advantageous when organs, in particular difficult to reach organs (e.g., heart, spleen, lung or liver) are the targeted sites of treatment.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., *Proc. Natl. Acad. Sci. USA* 189:11277-11281, 1992, which is incorporated herein by reference in its entirety). Oral delivery can be

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performed by complexing a therapeutic composition of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or

5   tablets, such as those known in the art. Topical delivery can be performed by mixing a therapeutic composition of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Therapeutic compositions of the present invention can

10   be administered to any animal, preferably to mammals and birds, and more preferably to humans, house pets, economic produce animals and zoo animals. Economic produce animals include animals to be consumed or that produce useful products (e.g., sheep for wool production). Zoo animals

15   include those animals harbored in zoos. Preferred animals to protect include humans, dogs, cats, sheep, cattle, horses and pigs, with humans and dogs being particularly preferred. While a therapeutic composition of the present invention is effective to treat disease in inbred species

20   of animals, the composition is particularly useful for treating outbred species of animals, in particular those having tumors.

Yet another embodiment of the present invention is a method to suppress T cell activity in an animal, the method

25   comprising administering to an animal an effective amount of a therapeutic composition comprising: (a) a naked nucleic acid molecule encoding a superantigen; and (b) a pharmaceutically acceptable carrier, in which the nucleic

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acid molecule is operatively linked to a transcription control sequence, and in which the therapeutic composition is targeted to a site in the animal that contains excessive T cell activity.

5   Suitable embodiments, single dose sizes, number of doses and modes of administration of a therapeutic composition of the present invention useful in a treatment method of the present invention are disclosed in detail herein.

10   A therapeutic composition of the present invention is also advantageous for the treatment of autoimmune diseases in that the composition suppresses the harmful stimulation of T cells by autoantigens (i.e., a "self", rather than a foreign antigen).   Superantigen-encoding recombinant

15   molecules in a therapeutic composition, upon transfection into a cell, produce superantigens that delete harmful populations of T cells involved in an autoimmune disease. A preferred therapeutic composition for use in the treatment of autoimmune disease comprises a superantigen-

20   encoding recombinant molecule of the present invention. A more preferred therapeutic composition for use in the treatment of autoimmune disease comprises a superantigen- encoding recombinant molecule combined with a non-targeting carrier of the present invention, preferably saline or

25   phosphate buffered saline.   Such   a   therapeutic composition of the present invention is particularly useful for the treatment of autoimmune diseases, including but not limited   to,   multiple   sclerosis,   systemic   lupus

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erythematosus, myasthenia gravis, rheumatoid arthritis, insulin dependent diabetes mellitus, psoriasis, polyarteritis, immune mediated vasculitides, immune mediated glomerulonephritis, inflammatory neuropathies and
5   sarcoidosis.

A single dose of a superantigen-encoding nucleic acid molecule in a non-targeting carrier to administer to an animal to treat an autoimmune disease is from about 0.1 μg to about 200 μg of total recombinant molecules per kilogram
10 (kg) of body weight, more preferably from about 0.5 μg to about 150 μg of total recombinant molecules per kg of body weight, and even more preferably from about 1 μg to about 10 μg of total recombinant molecules per kg of body weight.

The number of doses of a superantigen-encoding
15 recombinant molecule in a non-targeting carrier to be administered to an animal to treat an autoimmune disease is an injection about once every 6 months, more preferably about once every 3 months, and even more preferably about once a month.

20   A preferred method to administer a therapeutic composition of the present invention to treat an autoimmune disease is by local administration, preferably direct injection. Direct injection techniques are particularly important in the treatment of an autoimmune disease.
25 Preferably, a therapeutic composition is injected directly into muscle cells in a patient, which results in prolonged expression (e.g., weeks to months) of a recombinant molecule of the present invention. Preferably, a

recombinant molecule of the present invention in the form of "naked DNA" is administered by direct injection into muscle cells in a patient.

Another aspect of the present invention is an adjuvant for use with a nucleic acid-based vaccine to protect an animal from a disease or a remedy to treat a diseased animal. Adjuvants of the present invention comprise: (a) a superantigen-encoding nucleic acid molecule of the present invention; or (b) a combination of a superantigen-encoding nucleic acid molecule of the present invention with a cytokine nucleic acid molecule of the present invention, a chemokine nucleic acid molecule of the present invention or mixtures thereof.

Suitable compounds to combine with an adjuvant of the present invention, to form an adjuvant composition (i.e., a vaccine composition useful as a preventative therapeutic reagent or a therapeutic remedy useful to alleviate a disease) of the present invention, include any compound that is administered to an animal as an immunogen. As used herein, an immunogen of the present invention comprises a compound capable of eliciting an immune response in an animal. Preferably, an immunogen of the present invention is derived from a foreign agent including a pathogen. Also preferably, an immunogen of the present invention includes an allergen (organic or inorganic), tumor antigens and self-antigens.

A preferred immunogen is derived from a pathogen including, but not limited to, a virus, a bacteria, a

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eukaryotic parasite and unicellular protozoa (e.g., amoeba). Preferred eukaryotic parasites include protozoan parasites, helminth parasites (such as nematodes, cestodes, trematodes, ectoparasites and fungi.

5   A preferred immunogen also includes an allergen including, but not limited to, a plant allergen, an animal allergen, a bacterial allergen, a parasitic allergen, a metal-based allergen that causes contact sensitivity and inorganic allergens such as silica, beryllium, xenobiotics,
10   synthetic drugs and dyes. A more preferred allergen includes weed, grass, tree, peanut, mite, flea, cat, house dust and bacterial products antigens.

A preferred immunogen derived from a bacteria includes an immunogen that protects an animal from or alleviates
15   *Mycobacterium* infection, in particular *M. tuberculosis*, *M. leprae*, *M. avium*, and/or *M. bovis* infection. A more preferred bacterial immunogen of the present invention includes a peptide, mimetopes thereof and compositions containing the same, as disclosed in U.S. Patent Serial No.
20   08/484,169, filed June 7, 1995, which is incorporated herein by this reference. In one embodiment, an immunogen comprises a nucleic acid molecule encoding an immunogenic protein. Such immunogen-encoding nucleic acid molecules can be designed by those of skill in the art
25   based upon the amino acid sequence of the immunogen. In addition, a recombinant molecule encoding an immunogen of the present invention can be produced using the recombinant DNA technology disclosed herein and known to those of skill

in the art.   In other embodiments, an immunogen can comprise a peptide, a polypeptide or a chemical compound as disclosed herein.   All such embodiments of an immunogen are useful with an adjuvant of the present invention.

5   In order to treat an animal (i.e., vaccinate or remedy), an adjuvant composition of the present invention is administered to the animal in an effective manner such that the composition is capable of protecting an animal from or alleviating a disease.   For example, an adjuvant,

10 when administered to an animal in an effective manner, is able to stimulate effector cell immunity in a manner that is sufficient to prevent an initial or continued disease response by the subject animal.

An   effective   administration   protocol   (i.e.,

15 administering an adjuvant composition in an effective manner) comprises suitable dose parameters, and modes and times of administration that result in the treatment of an animal.   Effective dose parameters and modes of administration can be determined using methods standard in

20 the art for a particular adjuvant composition.   Such methods include, for example: determination of side effects (i.e., toxicity) of an adjuvant composition; progression of a disease upon administration of an adjuvant composition; magnitude and/or duration of antibody response by an animal

25 against an immunogen contained in an adjuvant composition; magnitude and/or duration of a cell mediated immune response in an animal against an adjuvant composition; similarity of an immune response to an adjuvant composition

in different species of animals; and/or effect of breed (in non-human animals) or race (in humans) on responsiveness to an adjuvant composition. In particular, the effectiveness of dose parameters and modes of administration of an

5   adjuvant composition of the present invention can be determined by assessing antibody production *in vivo*, skin test sensitivities *in vivo*, cytokine production *in vitro*, antigen-specific proliferation *in vitro*, cytotoxic T cell activity *in vitro*, reduction of tumor burden *in vivo* and/or

10 reduction of infectious agent burden *in vivo*. Tests standard in the art can be used to determine antibody production (e.g., enzyme-linked immunoassays), skin test sensitivities (e.g., subcutaneous injection of an immunogen into a vaccinated animal to assess weal formation,

15 induration and erythema), cytokine production (e.g., immunoassays using cytokine-specific antibodies or bio-assays using cytokine-dependent cell lines), antigen-specific proliferation (e.g., ^{3}H-thymidine incorporation), cytotoxic T cell activity (e.g., measure release of ^{51}Cr

20 from target cells), reduction of tumor burden (e.g., measure size of a tumor) and/or reduction of infectious agent burden (e.g., obtaining, for example, viral titers, bacterial colony counts or parasite counts).

An effective dose refers to a dose capable of

25 immunizing an animal against an immunogen. Effective doses can vary depending upon, for example, the adjuvant used, the immunogen being administered, and the size and type of the recipient animal. Effective doses to treat an animal

to an immunogen include doses administered over time that are capable of preventing or alleviating a disease in an animal to, for example, a pathogen or allergen. For example, a first treatment dose can comprise an amount of

5   an adjuvant composition of the present invention that causes a minimal hypersensitive response when administered to a hypersensitive animal. A second treatment dose can comprise a greater amount of the same adjuvant composition than the first dose. Effective treatment doses can comprise

10   increasing concentrations of the adjuvant composition necessary to treat an animal such that the animal does not exhibit signs of a disease.

In accordance with the present invention, a suitable single dose is a dose that is capable of vaccinating an

15   animal against a foreign agent when administered one or more times over a suitable time period. For example, a preferred single dose of an adjuvant composition of the present invention is from about 100 μg to about 1 mg of the adjuvant composition per kilogram body weight of the

20   animal. Further treatments with the adjuvant composition can be administered from about 1 week to about 1 year after the original administration. Further treatments with the adjuvant composition preferably are administered when the animal is no longer protected from an immunogen to which

25   the animal has been treated. Particular administration doses and schedules can be developed by one of skill in the art based upon the parameters discussed above.

The number of doses administered to an animal is dependent upon the immunogen and the response of an individual patient to the adjuvant composition. For example, treatment of one strain of virus may require more

5   doses than treatment of a more immunogenic strain of virus. Thus, it is within the scope of the present invention that a suitable number of doses includes any number required to treat an animal. A preferred number of doses of an adjuvant composition comprising a superantigen-encoding recombinant

10 molecule, and/or a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule is from about 2 to about 20 administrations, preferably from about 3 to about 10 administrations, and even more preferably from about 3 to about 5 administrations per patient per

15 year. Preferably, such administrations are given once every 2 weeks until, for example, antibody production against an immunogen increases or decreases, cell mediated immunity increases, and/or a clinical response is observed when an adjuvant composition is administered as a therapeutic

20 remedy.

A preferred single dose of the superantigen-encoding recombinant molecule is an amount that, when transfected into a muscle cells, skin tissue, lung cells or other suitable cellular sites, leads to the production of from

25 about 10 femtograms (fg) to about .01 μg, preferably from about 100 fg to about 1 picogram (pg), and more preferably from about 1 pg to about 5 pg of superantigen per transfected cell. A preferred single dose of a cytokine-

encoding recombinant molecule is an amount that when
transfected into a target cell population leads to the
production of from about 10 pg to about .01 µg, preferably
from about 100 fg to about 2 pg, and more preferably about

5   1 pg of cytokine per transfected. A preferred single dose
of a chemokine-encoding recombinant molecule is an amount
that when transfected into a target cell population leads
to the production of from about 1 pg to about .01 µg,
preferably from about 0.1 pg to about 10 pg, and more

10   preferably about 1 pg of chemokine per transfected.

In one embodiment, an adjuvant composition of the
present invention comprises up to about 50% of an
immunogen-encoding recombinant molecule and up to about 50%
of a superantigen-encoding recombinant molecule.

15   Preferably, an adjuvant composition of the present
invention comprises no more than about 1.5 mg of immunogen-
encoding recombinant molecule and no more than about 1.5
mg of superantigen-encoding recombinant molecule, more
preferably no more than about 1 mg of immunogen-encoding

20   recombinant molecule and no more than about 1 mg of
superantigen-encoding recombinant molecule, and even more
preferably no more than about 0.5 mg of immunogen-encoding
recombinant molecule and no more than about 0.5 mg of
superantigen-encoding recombinant molecule per animal.

25   In another embodiment, an adjuvant composition of the
present invention comprises an immunogen-encoding
recombinant molecule in an amount up to about 66% by weight
of the composition and a superantigen-encoding recombinant

molecule in an amount up to about 33% by weight of the composition. Preferably, an adjuvant composition of the present invention comprises no more than about 2000 µg of immunogen-encoding recombinant molecule and no more than

5 about 1000 µg of superantigen-encoding recombinant molecule, more preferably no more than about 1400 µg of immunogen-encoding recombinant molecule and no more than about 660 µg of superantigen-encoding recombinant molecule, and even more preferably no more than about 670 µg of

10 immunogen-encoding recombinant molecule and no more than about 330 µg of superantigen-encoding recombinant molecule per animal.

In another embodiment, an adjuvant composition of the present invention comprises an immunogen-encoding

15 recombinant molecule in an amount up to about 50% of the composition; a superantigen-encoding recombinant molecule in an amount up to about 25% of the composition; and a cytokine-encoding recombinant molecule or chemokine-encoding recombinant molecule or mixtures thereof, in an

20 amount up to about 25% of the composition. According to the present embodiment, a cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule can be used alone or in combination with each other. When used in combination, the ratio of cytokine-encoding recombinant

25 molecule to chemokine-encoding recombinant molecule can be varied according to need. The ratio can be determined based upon the effectiveness of the adjuvant composition at

vaccinating an animal against a foreign agent using the methods and parameters disclosed herein.

In one embodiment, an adjuvant composition of the present invention comprises: no more than about 2000 μg of immunogen-encoding recombinant molecule, no more than about 500 μg of superantigen-encoding recombinant molecule, and no more than about 500 μg of cytokine-encoding recombinant molecule or no more than about 500 μg of chemokine-encoding recombinant molecule; more preferably no more than about 1400 μg of immunogen-encoding recombinant molecule, no more than about 300 μg of superantigen-encoding recombinant molecule, and no more than about 300 μg of cytokine-encoding recombinant molecule or no more than about 300 μg of chemokine-encoding recombinant molecule; and even more preferably no more than about 660 μg of immunogen-encoding recombinant molecule, no more than about 160 μg of superantigen-encoding recombinant molecule, and no more than about 160 μg of cytokine-encoding recombinant molecule or no more than about 160 μg of chemokine-encoding recombinant molecule per animal.

In another embodiment, an adjuvant composition of the present invention comprises: no more than about 2000 μg of immunogen-encoding recombinant molecule, no more than about 500 μg of superantigen-encoding recombinant molecule, and no more than about 250 μg of cytokine-encoding recombinant molecule and no more than about 250 μg of chemokine-encoding recombinant molecule; more preferably no more than about 1000 μg of immunogen-encoding recombinant molecule,

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no more than about 250 μg of superantigen-encoding
recombinant molecule, and no more than about 125 μg of
cytokine-encoding recombinant molecule and no more than
about 125 μg of chemokine-encoding recombinant molecule;
5   and even more preferably no more than about 660 μg of
immunogen-encoding recombinant molecule, no more than about
160 μg of superantigen-encoding recombinant molecule, and
no more than about 80 μg of cytokine-encoding recombinant
molecule and no more than about 80 μg of chemokine-encoding
10 recombinant molecule per animal.

Adjuvant compositions are preferably delivered by
intramuscular administration in the form of "naked" DNA
molecules, such as disclosed herein.   Preferably, an
adjuvant composition of the present invention is delivered
15 by intramuscular, intravenous, intraperitoneal and/or
intraarterial injection, and/or injection directly into
specific cellular locations (e.g., into a tumor).
Preferred sites of intramuscular injections include caudal
thigh muscle, back muscle and into the buttocks of a human.

20

Preferably, an adjuvant composition of the present
invention comprises a suitable pharmaceutically acceptable
carrier for delivering the composition intramuscularly. A
preferred carrier for use with an adjuvant includes
25 phosphate buffered saline, water, Ringer's solution,
dextrose solution, Hank's balanced salt solution and normal
saline.   A more preferred carrier includes phosphate

buffered saline and normal saline, with phosphate buffered saline being even more preferred.

Preferably, an adjuvant composition of the present invention comprises a mixture including a superantigen
5   encoding molecule including an SEA-encoding recombinant molecule, an SEB-encoding recombinant molecule or mixtures thereof, and an immunogen-encoding recombinant molecule of the present invention; a superantigen encoding molecule including an SEA-encoding recombinant molecule, an SEB-
10   encoding recombinant molecule or mixtures thereof, a cytokine encoding molecule including a GM-CSF-encoding recombinant molecule and an immunogen-encoding recombinant molecule of the present invention; or a superantigen encoding molecule including an SEA-encoding recombinant
15   molecule, an SEB-encoding recombinant molecule or mixtures thereof, a chemokine encoding molecule including a MIP1α, MIP1β, IL-8 or RANTES recombinant molecule and an immunogen-encoding recombinant molecule of the present invention.

20   In a preferred embodiment, an adjuvant of the present invention includes the following recombinant molecules contained in phosphate buffered saline: (1) PCR$_3$-SEA, PCR$_3$-SEA.S, PCR$_3$-SEB, PCR$_3$-SEB.S, PCR$_3$-TSST and mixtures thereof; (2) mixtures of up to about 50% PCR$_3$-SEA, PCR$_3$-SEA.S, PCR$_3$-
25   SEB, PCR$_3$-SEB.S and/or PCR$_3$-TSST, and up to about 50% PCR$_3$-GM$_3$; (3)
mixtures of up to about 50% PCR$_3$-SEA, PCR$_3$-SEA.S, PCR$_3$-SEB, PCR$_3$-SEB.S and/or PCR$_3$-TSST, and up to about 50% PCR$_3$-MIP1α;

(4) mixtures of up to about 50% PCR$_3$-SEA, PCR$_3$-SEA.S, PCR$_3$-SEB, PCR$_3$-SEB.S and/or PCR$_3$-TSST, and up to about 50% PCR$_3$-MIP1β; (5) mixtures of up to about 50% PCR$_3$-SEA, PCR$_3$-SEA.S, PCR$_3$-SEB, PCR$_3$-SEB.S and/or PCR$_3$-TSST, and up to about 50%

5   PCR$_3$-RANTES; (6) mixtures of up to about 50% PCR$_3$-SEA, PCR$_3$-SEA.S, PCR$_3$-SEB, PCR$_3$-SEB.S and/or PCR$_3$-TSST, up to about 25% PCR$_3$-GM$_3$, and up to about 25% PCR$_3$-MIP1α, PCR$_3$-MIP1β and/or PCR$_3$-RANTES.

According to the present invention, a preferred

10   embodiment of an adjuvant composition of the present invention includes: (1) an immunogen-encoding recombinant molecule the present invention in an amount up to about 50% of the composition and a preferred embodiment of an adjuvant of the present invention in an amount up to about

15   50% of the composition; or (2) an immunogen-encoding recombinant molecule in an amount up to about 66% of the composition and a preferred embodiment of an adjuvant of the present invention in an amount up to about 33% of the composition, in phosphate buffered saline.

20   The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

Example 1

25   This example describes the production of recombinant molecules 'encoding superantigens and cytokines.

Full-length cDNA encoding *Staphylococcal enterotoxin* B (SEB; SEQ ID NO:1) and *Staphylococcal enterotoxin* A (SEA;

SEQ ID NO:3) were produced by polymerase chain reaction
(PCR) amplification using templates obtained from Dr. John
Kappler (National Jewish Center for Immunology and
Respiratory Disease, Denver, CO). A truncated form of SEB

5   lacking the leader sequence, which spans base pairs 46 to
773 (referred to herein as SEB.S), was prepared by PCR
amplification   using   the   primers   5'
GGGAATTCCATGGAGAGTCAACCAG   3'   (SEQ   ID   NO:7)   and   3'
GCGGATCCTCACTTTTTCTTTGT 5' (SEQ ID NO:8). A truncated form

10   of SEA lacking the signal sequence, which spans base pairs
46 to 751 (referred to herein as SEA.S), was prepared by
PCR   amplification   using   the   primers   5'
GGGAATTCCATGGAGAGTCAACCAG   3'   (SEQ   ID   NO:9)   and   5'
GCAAGCTTAACTTGTATATAAATAG 3'(SEQ ID NO:10). Full-length

15   cDNA encoding Toxic Shock Syndrome Toxin (TSST; SEQ ID
NO:5) was produced by PCR amplification using a template
obtained from Dr. Brian Kotzin (National Jewish Center for
Immunology and Respiratory Disease, Denver, CO), using the
primers:

20   5' CGGGGTACCCCGAAGGAGGAAAAAAAAATGTCTACAAACGATAATATAAAG 3'
(SEQ ID NO:11); and

3' TGCTCTAGAGCATTAATTAATTTCTGCTTCTATAGTTTTTAT 5' (SEQ ID
NO:12).

      Each cDNA clone was ligated into the eukaryotic

25   expression vector PCR$_3$ (In Vitrogen, San Diego, CA) using
standard cloning methods. The full-length SEB cDNA cloned
into PCR$_3$ is referred to herein as PCR$_3$-SEB; the full-length
SEA cDNA cloned into PCR$_3$ is referred to herein as PCR$_3$-SEA;

the full-length TSST cDNA cloned into PCR$_3$ is referred to herein as PCR$_3$-TSST; the truncated SEB cDNA cloned into PCR$_3$ is referred to herein as PCR$_3$-SEB.S; and the truncated SEA cDNA cloned into PCR$_3$ is referred to herein as PCR$_3$-SEA.S.

5   A cDNA for canine GM-CSF was produced by PCR amplification of total RNA extracted from Concavalin A-stimulated normal canine peripheral blood mononuclear cells (PBMC) using canine GM-CSF primers designed based on the published canine GM-CSF cDNA (Nash, *ibid.*). The total RNA

10   was reverse transcribed using the reverse transcriptase enzyme and oligoT primers. The canine GM-CSF cDNA was then amplified using PCR and specific 5' and 3' primers. The PCR product was cloned into the PCR$_3$ vector, the resulting recombinant molecule is referred to herein as PCR$_3$-GM$_3$.

15   **Example 2**

This example describes the expression of DNA encoding superantigens in mammalian CHO cells following transfection.

Isolated PCR$_3$-SEB.S, PCR$_3$-SEA.S and PCR$_3$-TSST were

20   transformed into *E. coli* cells and ampicillin-resistant bacterial colonies were screened for the presence of the plasmid. Selected colonies were then cultured in large scale culture (liter volume). Plasmid DNA was isolated using standard methods. A typical plasmid yield was 20 mg

25   plasmid DNA from one liter of bacteria-containing culture medium. Plasmid DNA was transfected into Chinese hamster ovary cells (CHO) by lipofection (Lipofectamine, Gibco-BRL, Gaithersburg, MD) using methods provided by the

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manufacturer.   About 2.0 μg of each plasmid DNA was separately transfected into about 10^6 CHO cells.

   The transfected CHO cells were cultured for 48 hours. Supernatants and cell lysates were then isolated to
5 determine the amount of intracellular and secreted SAg protein produced by the transfected cells. Cell lysates were prepared by detaching and sonicating the transfected cells to prepare cell lysates to measure activity. SAg protein activity in each sample was measured by
10 quantitating the ability of the SAg protein to stimulate lymphocyte contained in a PBMC population using the following method. Supernatants and lysates to be tested were added in serial dilutions to triplicate wells of a 96-well plate containing 5 X 10^5 PBMC in a total volume of 200
15 μl per well. After 3 days, the wells were pulsed with ^{3}H thymidine and incubated for 18 hours. The radioactivity incorporated into the PBMC's were quantitated on a beta counter. Negative controls included CHO cells transfected with the DNA vector without an inserted gene (mock) and
20 positive controls were purified recombinant SAg proteins.

   The results were plotted as the mean incorporated thymidine in counts per minute and are shown in Fig. 1. The results indicate that both supernatants and lysates of
25 CHO cells transfected with PCR$_3$-SEB.S, PCR$_3$-SEA.S and PCR$_3$-TSST stimulated strong proliferation of the PBMC's, compared to mock transfected cultures. The activity in supernatants in some cases exceeded that in cell lysates.

Thus, DNA encoding bacterial SAg proteins are capable of being transcribed and translated in mammalian cells in biologically active form. The results also indicate that the amounts of biologically active SAg protein are active

5   produced by the transfected cells was sufficient to stimulate T cell proliferation.

Example 3

This example describes the expression of DNA encoding superantigens in canine melanoma cells following

10   transfection.

A melanoma cell line was established from an oral malignant melanoma obtained by biopsy from a canine patient by isolating a portion of a melanoma tumor, digesting that portion with collagenase and plating the released cells in

15   24 well plates using Iscove Modified Dulbecco's Medium (IMDM) with 10% fetal calf serum. Melanoma cells were transfected with PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST by lipofection as described in Example 2. The cells were then irradiated (15,000 Rads). Four samples of each sample of

20   transfected melanoma cells were prepared, in which decreasing numbers of the transfected cells were added to normal canine PBMC (5 X 10^5/well). Each sample was prepared in triplicate in a 96 well plate. After 3 days, proliferation was quantitated as described in Example 2.

25   Non-transfected melanoma cells were used as negative control samples. The results were plotted as the mean incorporated thymidine in counts per minute and are shown in Fig. 2. The results indicate that Canine PBMC

proliferated when cultured with canine melanoma cells transfected with PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST, exhibiting a dose-dependent increase in proliferation as increasing numbers of irradiated tumor cells were used.

5   Thus, melanoma tumor cells can be transfected and can express biologically active SAg protein. The results also show that the transfected melanoma cells continue to release biologically active SAg protein after irradiation, indicating that transfected tumor cells would also be

10   useful as an autologous tumor vaccine as described in detail in the present application.

Example 4

This example describes the long term expression of DNA encoding SEB.S and SEA.S in stably transfected CHO cells.

15   To determine whether the SAg protein activity detected in supernatants of transfected CHO cells (described in Example 2) represented actual secretion or simple release from dying cells, stably transfected CHO lines were prepared using either PCR_3-SEB.S, PCR_3-SEA.S or vector with

20   no cDNA insert (control). About 2 x 10^6 CHO cells were transfected with about 2 μg of plasmid DNA by lipofection. The transfected cells were then cultured in G418 (1 mg/ml) for 3 weeks to obtain stable transfectants. The G418 selected CHO cells were seeded into 9 individual tissue

25   culture wells, allowed to adhere for 4 hours, and then fresh tissue culture media was added. Supernatants were harvested sequentially, beginning at time zero and continuing for 36 hours. Supernatants were added to PBMC

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to assay for SAg protein activity, as described in Example 2.

The results were plotted as the mean proliferation stimulating activity contained in supernatants at each time

5   point and are shown in Figs. 3A and 3B. The results indicate that a steady time-dependent increase in PBMC stimulatory activity was observed in supernatants from CHO cells stably transfected with both PCR_3-SEB.S and PCR_3-SEA.S. Thus, transfection of mammalian cells with PCR_3-

10   SEB.S, PCR_3-SEA.S results in long term expression of biologically active SAg protein. The data indicates that transfected mammalian cells can serve as a sustained source of SAg protein production.

Example 5

15   This example describes that transfection of PCR_3-SEA.S DNA in melanoma cells results in the expression of biologically active SEA.S protein.

Superantigens are capable of stimulating the proliferation of T cells bearing certain Vβ domains in

20   their T cell receptor (TCR). SEA is known to stimulate T cells having a Vβ3+ TCR in mice. SEB does not stimulate Vβ3+ T cells. Therefore, an experiment was performed to assess the ability of SEA.S protein expressed by melanoma cells transfected with PCR_3-SEA.S DNA to stimulate the

25   proliferation of a T cell clone (AD10) expressing the Vβ3+TCR.

B16 melanoma cells were transfected with PCR_3-SEA.S DNA, PCR_3-SEB.S or PCR_3 vector DNA with no insert (mock).

The cells were then irradiated (18,000 Rads) and plated in triplicate in a 96 well plate at a concentration of about 1×10^4 per well. About 1×10^5 AD10 cells were added to each well. Next, irradiated syngeneic spleen cells were

5 added to each well as a source of antigen presenting cells for the superantigen and T cell interaction. Negative controls included mock transfected cells; positive controls included recombinant SEA (10ng/ml). The cells were incubated for 48 hours. ^{3}H thymidine was then added to each

10 well and the proliferative response quantitated.

The results were plotted as the mean incorporated thymidine in counts per minute and are shown in Fig. 4. The AD10 cells proliferated strongly in response to SEA.S protein produced by the PCR_3-SEA.S DNA transfected into the

15 B16 cells, with the proliferative response nearly equal to that of the recombinant protein. Thus, the T cell response generated by transfection of melanoma cells with PCR_3-SEA.S DNA is specific for the correct TCR. Cells transfected with PCR_3-SEB.S DNA did not stimulate proliferation of AD10

20 cells, consistent with the predicted TCR specificity of SEA and SEB.

Example 6

This example describes the expression of PCR_3-GM DNA in CHO cells.

25   PCR_3-GM DNA was produced, isolated and transfected into CHO cells using the methods described in Examples 1 and 2. Expression of GM-CSF protein in the CHO cells was measured by the following method. Supernatants were isolated from

the cultures of the transfected cells and non-transfected CHO cells. The supernatants were added to cultures of monocyte cells (obtained from normal canine PBMC) and the ability of the supernatants to support the growth and
5   survival of monocytes was determined. After 4 days in culture with test or control CHO supernatants, monocyte survival was quantitated by addition of methyltetrazolium dye (MTT) that is reduced in viable cells. Absorbance of light at 570 nm (measured using an ELISA reader) is
10 representative of cell survival.

The results are shown in Fig. 5 and indicate that the supernatants from CHO transfected with PCR$_3$-GM DNA stimulated the survival of canine monocytes in culture compared with results obtained using the control
15 supernatants. The level of activity was comparable to that of 1×10^5 units of canine recombinant GM-CSF. Thus, the GM-CSF protein produced by CHO cells transfected with PCR$_3$-GM DNA is biologically active.

Example 7
20   This example demonstrates that the vaccination of mice with autologous tumor cells transfected with PCR$_3$-SEA.S DNA or PCR$_3$-SEB.S DNA induce strong cytotoxic T cell (CTL) activity.

The following experiment studies the ability of non-
25 immunogenic murine melanoma cells (B16 melanoma cells, F10 clone) expressing either PCR$_3$-SEA.S DNA or PCR$_3$-SEB.S to induce CTL responses in mice. B16 cells are known to be non-immunogenic when injected into C57B16/J mice. The

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level of CTL responses that can be induced has been shown
to correlate with the ability of the immunized animal to
reject tumors.

B16 cells were transfected with either PCR$_3$-SEA.S DNA,
5   PCR$_3$-SEB.S or PCR$_3$ DNA lacking insert (mock) using the
method described in Example 2.   The cells were then
irradiated at 12,000 Rads. About 10^6 irradiated cells were
then injected subcutaneously into C57B16/J mice.   Three
weeks later, the mice were sacrificed and their spleen
10 mononuclear cells harvested.   Mononuclear cells isolated
from the spleen cells were then restimulated *in vitro* with
irradiated, non-transfected wild type B16 cells for 6 days
in media with interleukin-2 (IL-2).   The spleen cells were
then added in decreasing numbers to about 5×10^3 of ^{51}Cr-
15 labeled wild type (non-transfected) B16 cells in a standard
chromium release assay for CTL activity.   After 4 hours,
the supernatants were harvested and the percentage of
specific lysis of the target B16 melanoma cells was
quantitated.

20   The results are shown in Figs. 6A and 6B and indicate
that injection of animals with irradiated transfected
melanoma cells induce greater CTL activity than injection
with non-transfected cells.   This result is consistent with
the non-immunogenic nature of B16 cells.   Thus, DNA
25 encoding bacterial SAg proteins expressed in transfected
tumor cells are capable of eliciting strong CTL-mediated
immunity against the non-transfected parental cell. These
results suggest that autologous tumor cells transfected

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with DNA encoding a superantigen constitute an effective tumor vaccine for treatment or prevention of metastatic disease.

Example 8

5   This example demonstrates that tumor cells transfected with PCR$_3$-SEB.S DNA are capable of inducing cytotoxic activity in adjacent T cells.

T cells were prepared from a mouse immunized with non-transfected B16 cells using the methods described in

10   Example 7. These isolated cells exhibited minimal CTL activity towards non-transfected B16 target cells. B16 cells were transfected with PCR$_3$-SEB.S using the methods generally described in Example 2. Induction of CTL activity by the transfected B16 target cells was assessed

15   in a standard 4 hour chromium release assay as used in Example 7.

The results are shown in Fig. 7 and indicates that B16 cells transfected with PCR$_3$-SEB.S produced protein that rapidly induced a four-fold increase in CTL activity in T

20   cells that were relatively unresponsive to non-transfected target B16 cells. Thus, the SEB produced in the vicinity of the isolated T cells by the B16 cells is capable of stimulating such T cells. The data indicates that tumor cells transfected *in vivo* with PCR$_3$-SEB.S can produce

25   biologically active SEB.S that is capable of rapidly activating' T lymphocytes in their vicinity and thereby inducing cytotoxic activity against themselves or neighboring tumor cells.

Example 9

    This example describes the treatment of canine melanoma with DNA encoding superantigen or GM-CSF.

A.   Criteria for entry and trial design

5   Animals selected for entry into the present study were client owned animals with spontaneous oral malignant melanoma, a highly malignant neoplasm of dogs for which there is no alternative effective treatment. Prior to entry, the owners were required to sign informed consent. 10 The study consisted of an initial 12 week trial response phase with 6 injections given once every 2 weeks, followed by long term once monthly maintenance therapy for those animals that responded during the initial 12 week induction phase. Potential toxicity was assessed by (1) body 15 temperature measured daily for 7 days after injection; (2) physical examination of the injection site; (3) owner's assessment of their pet's attitude and appetite; (4) complete blood counts and biochemistry measurements once monthly. Treatment responses were assessed by: (1) 20 physical measurement of tumor dimensions; (2) tumor photography; (3) thoracic radiographs for metastasis evaluation.

B.   Superantigen + GM-CSF Treatment protocol

    DNA samples complexed with liposomes were prepared as 25 follows. PCR$_3$-SEB.S and PCR$_3$-GM plasmid DNA prepared from bacterial cultures by the alkaline lysis method, then purified by CsCl banding, were resuspended at a 1.0 mg/ml concentration in sterile PBS. Liposomes were prepared by

mixing equimolar amounts of N-[1-(33-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA; obtained from Syntex, Corp., Palo Alto, CA) and dioleoyl phosphatidylethanolamine (DOPE; obtained from Avanti Polar Lipids, Birmingham, AL). The

5   lipids were dried in a desiccator and reconstituted at a concentration of 1.0 mg/ml in sterile phosphate buffered saline (PBS), pH 7.0. The reconstituted lipids were sonicated for 5 minutes to produce liposomes having an average size of about 200 nm to about 400 nm.

10   Thirty minutes prior to injection into the patients, the PCR_3-SEB.S and PCR_3-GM DNA was mixed with the liposomes at a ratio of 1.0 μg DNA to 4 nmol liposome, in 1.0 ml sterile PBS. The solution was allowed to complex at room temperature. Two doses of DNA were administered, depending

15   on tumor volume. For tumors less than 3 centimeters (cm) in diameter, 400 μg total DNA (200 μg each of PCR_3-SEB.S and PCR_3-GM DNA) were injected into each tumor. For tumors larger than 3 cm diameter, a total of 800 μg DNA (400 μg each of PCR_3-SEB.S and PCR_3-GM DNA) were injected into each

20   tumor.

For each treatment, the DNA:liposome mixture was injected into the tumor site with a 3 ml syringe and 25 gauge needle. For larger tumors, most of the injection was delivered into tissues at the periphery of the tumor base.

25   For some smaller tumors, injections were also injected directly into tumor tissue. Lymph node tissue having evidence of tumor metastasis was also injected. Injections were performed once every 2 weeks for the first 12 weeks,

then continued twice monthly for those animals in which an
initial treatment response occurred, until complete tumor
regression occurred.   At that time, the frequency of
injections decreased to once monthly.   The toxicity of the
5   treatment was evaluated based on the parameters outlined
above in section A. The results are shown below in Table 1.

Table 1.   Patient Log for SEB.S and PCR₃-GM DNA Treatment of Canine Melanoma

Patient	Stage	TN	Tumor Size	Start Date	Response	Comments
Zomax	I	T1bNOMO	1.5 cm diam	5/16/94	CR 51 wks	SEB.S + GM-CSF
Shadow	III	T2bN1bMO	3 cm diam	5/23/94	CR 50 wks	SEB.S + GM-CSF
NG	I	T1NOMO	1.2 cm diam	9/12/94	CR 34 wks	SEB.S + GM-CSF
Maggie	II	T2aNOMO	2 cm diam	8/24/94	PR 33 wks	SEB.S + GM-CSF
K.C.	III	T3aNOMO	> 4 cm diam	10/13/94	SD 12 wk	SEB.S + GM-CSF
Belvedere	III	T2N1bMO	4 cm diam	10/13/94	CR 30 wks	SEB.S + GM-CSF
Nicholas	III	T3bNOMO	> 4 cm diam	2/15/95	SD 12 wks	SEB.S + GM-CSF
Heidi	III	TON1bMO	LN:2cm diam	2/27/95	PR 10 wks	SEB.S + GM-CSF
Bear	III	TON1bMO	LN:2.5cm	4/11/95	SD 4 wks	SEB.S + GM-CSF

<u>Key to terminology in patient data sheets:</u>

Stage: I represents the smallest and III the largest size, with metastases
TNM: World Health Organization staging system
SD   =   stable disease (no tumor growth)
PR   =   partial remission (> 50% decrease in tumor size)
CR   =   tumor completely regressed
PD   =   progressive disease, no response to treatment
MCT   =   mast cell tumor
Mammary CA = mammary gland adenocarcinoma (malignant breast cancer)
Thyroid CA = thyroid adenocarcinoma
SCC   = squamous cell carcinoma

The results shown in Table 1 indicate that a treatment response was observed in 6 of 9 dogs treated for the 12 week trial period. This included 4 complete remissions (no residual tumor) and 2 partial remissions (greater than 50%

5   reduction in tumor size). Tumors in the remaining two dogs did not regress, but also did not progress in size during the 12 week trial. On average, a tumor response required 6 to 10 weeks to become apparent. The injections did not cause any inflammation or necrosis at injections sites.

10   Toxicity, either local or systemic, was not observed in any of the 10 patents treated in this study. These results provide evidence of the efficacy of direct DNA injection using DNA encoding superantigen (SEB) and cytokine (GM-CSF) for treatment of spontaneous malignant melanoma in an

15   outbred species.

Canine melanoma is a highly malignant, rapidly growing tumor of dogs, and provides a useful model for the study of treatments for human melanoma. Without treatment, the 50% survival time for animals with stage III disease (5 of the

20   patients in this study) is about 3 months and all animals will be dead by 5 months due to pulmonary metastases. The observation of several long term survivors shown in Table 1 (others have not been treated long enough to evaluate) suggests that the combined DNA immunotherapy approach also

25   has a systemic effect on preventing metastatic diseases.

Another major advantage of this approach is the apparent complete absence of toxicity in the dogs. Since dogs respond to SAg protein similar to humans, it is also

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likely that toxicity in humans would also be minimal. The delivery of DNA encoding superantigens into tumor cells by transfection and subsequent local expression is sufficient to induce a strong immune response without inducing
5   toxicity.   Thus this genetic approach to tumor immunotherapy offers advantages over conventional chemotherapy and radiation therapy in terms of reducing patient morbidity. In addition, delivering the SAg protein by DNA transfection also avoids the potential toxicity
10   associated with systemic administration.

C.   Single Gene Treatment Protocol

To evaluate the effectiveness of injecting DNA encoding either a superantigen or a cytokine, relative to combined genetic therapy (SAg-encoding DNA and cytokine-
15   encoding DNA), 2 groups of dogs were treated with either PCR$_3$-SEB.S DNA alone (3 dogs) or PCR$_3$-GM DNA alone (3 dogs; 2 entered, one evaluatable). Similar criteria for entry and trial design described above in Section A of this example was applied. Although not formally randomized,
20   after the first 10 dogs were treated with the 2 gene combination, the next 3 enrollees were assigned the PCR$_3$-SEB.S DNA alone group and the next 3 to the PCR$_3$-GM DNA alone group. A similar treatment protocol as described above in section B was applied in this study. Briefly, the
25   DNA was complexed with liposomes and injected once every 2 weeks for the first 12 weeks, then continued twice monthly for those animals in which an initial treatment response occurred, until complete tumor regression occurred. The

toxicity of the treatment was evaluated based on the parameters outlined above in section A. The results are shown below in Table 2.

Table 2. Patient Log for SEB.S or PCR$_3$-GM DNA alone Treatment of Canine Melanoma

Patient	Stage	TN	Tumor Size	Start Date	Response	Comments
Jessie	II	T2bNOMO	2 cm diam	1/11/95	PD 17 wks	SEB.S alone
Mr. T	III	TON1bMO	LN:2cm diam	2/1/95	PD 14 wks	SEB.S alone
Duffy	II	T2aNOMO	2 cm diam	2/3/95	PD 12 wks	SEB.S alone
Scooter	I	T2aNOMO	2 cm diam	3/24/95	PD 7 wks	GM-CSF alone

5

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The results indicated that a tumor response did not occur in any dog receiving PCR₃-SEB.S DNA alone and tumors grew progressively. In addition, one dog (Scooter) treated with PCR₃-GM DNA alone also exhibited progressive growth.

5   These data indicate that treatment with PCR₃-SEB.S DNA alone or PCR₃-GM DNA alone does not induce tumor regression. The data indicate that the marked anti-tumor efficacy of direct DNA injection results from the combined expression of PCR₃-SEB.S DNA and PCR₃-GM DNA in a tumor and adjacent tissues.

10 Example 10

This example describes the treatment of various tumor types with superantigen or GM-CSF encoding DNA.

The efficacy and lack of toxicity of PCR₃-SEB.S DNA and PCR₃-GM DNA was determined for the treatment of dogs with

15 malignancies having similar biological and histological characteristics as human cancers. Dogs with five different cancers (advanced mammary carcinoma, mast cell tumor, thyroid carcinoma, non-oral melanoma, and squamous cell carcinoma) were treated in this study. Animals selected

20 for entry into the present study included dogs with spontaneous malignancies that had received alternative treatments (e.g., chemotherapy and/or surgery) and either, had not responded, or had relapsed.

Therapeutic samples were prepared and injected

25 intratumorally with PCR₃-SEB.S DNA and PCR₃-GM DNA as described above in Example 2. The dogs were treated initially once every 2 weeks for 12 weeks, then continued twice monthly for those animals in which an initial

treatment response occurred. The toxicity of the treatment
was evaluated based on the parameters outlined above in
Example 9, section A. The results are shown below in Table
3.

Table 3. Patient Log for SEB.S and PCR$_3$-GM DNA Treatment of Various Carcinomas

Patient	Tumor Type	Stage	TN	Tumor Size	Start Date	Response	Comments
Emma	Mammary CA	III	T4N1bNMO	1.8 cm diam	8/11/94	PR 22 wks	SEB.S + GM-CSF
Baby	Mammary CA	II	T1aN1bMO	2.6 cm diam	9/12/94	PR 8 wks	SEB.S + GM-CSF
Christa	MCT	IIIa	NA	>2 cm diam	7/27/94	SD 39 wks	SEB.S + GM-CSF
Jack	MCT	IIIa	NA	>3 cm diam	3/28/95	PD 4 wks	SEB.S + GM-CSF
Britt	Thyroid CA	III	T3bNOMO	>7 cm diam	10/14/94	SD 16 wk	SEB.S + GM-CSF
Duncan	Melanoma Toe	NA*	T2N1MO	>4 cm diam	8/11/94	SD 20 wks	SEB.S + GM-CSF
Billy	Melanoma Toe	NA*	TON1bMO	LN 3.5 cm	1/10/95	CR 17 wks	SEB.S + GM-CSF
Scotche	SCC Tonsil	NA	T3NOMO	4 cm diam	3/27/95	SD	SEB.S + GM-CSF

*   Metastases
NA   Not Applicable
CA   Carcinoma
MCT   Mast Cell Tumor
SCC   Squamous Cell Carcinoma

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In this study, toxicity was not observed in any of the animals. Tumor responses (partial remission of the primary tumors) were observed in the animals with mammary carcinoma and neither animal developed additional metastatic disease

5   during the course of the study. Treatment of one dog (Billy) with a large, metastatic (lymph node metastases), non-oral melanoma resulted in complete remission of the cancer. Treatment of the other dog (Duncan) with a large, metastatic (lymph node metastases), non-oral melanoma

10 resulted in prolonged stabilization of the disease. The dog with thyroid cancer (Britt) also experienced prolonged stabilization of the disease with once monthly injections. The response rate for the dogs with mast cell tumors was low. The effectiveness of the treatment on the squamous

15 cell carcinoma is in early stages of evaluation. Taken together, the results indicate that PCR$_3$-SEB.S DNA and PCR$_3$-GM DNA can effectively treat multiple tumor types, in addition to the melanomas reported above in Example 9.

Example 11

20   This example describes the injection of PCR$_3$-SEA.S DNA into muscle cells which induces potent, long-lasting T cell deletion.

    Four groups of mice B10.BR (2-3 mice per group) were prepared as follows. Group (1) consisted of untreated mice

25 (control mice). Group (2) consisted of mice injected intraperitoneally with 100 ng of recombinant SEA (rSEA) protein.   Group (3) consisted of mice injected

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intramuscularly with 100 μg of PCR₃-SEA.S DNA (50 μg per leg, total of 100 μg/mouse). Group (4) consisted of mice injected intramuscularly with 100 μg PCR₃ (no insert; mock) DNA (50 μg per leg, total of 100 μg/mouse). The DNA samples

5   were prepared by diluting 100 μl of a solution containing 100 μg of DNA 50:50 (v:v) in sterile PBS prior to injection. The rSEA protein was purified from cultures of *E. coli* cells transformed with the recombinant molecule PKK223 (obtained from Dr. John Kappler) encoding the

10   truncated SEA.S protein lacking a leader sequence.

        Beginning 72 hours after injection, mice were tail bled and PBMC prepared for fluorescence activated cell sorter (FACS) analysis. Cells were double labeled with the monoclonal antibodies FITC conjugated-GK1.5 antibody,

15   biotinylated-KJ25 antibody and biotinylated-F23.1, to analyze for expression of CD4, TCR Vβ3 and TCR Vβ8 expression, respectively. The labelled cells were analyzed on an EPICS-C flow cytometer.

        The percentage of cells isolated from the experimental

20   mice expressing CD4 that also expressed either Vβ8 or Vβ3 was calculated and compared to percentages expressed by cells isolated from control mice. The mean percentage of CD4+ and Vβ3+ T cells in PBMC was plotted against time after injection. The results are shown in Fig. 8 and

25   indicate that the percentage of CD4+, Vβ3+ T cells declined rapidly in PBMC of mice that received intramuscular injections with PCR₃-SEA.S DNA, but not in mice mock

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injected with mock DNA. The percentages of Vβ8+ cells was
not affected. This result is predicted since SEA protein
does not bind mouse Vβ8+ T cells. The decline of the
percentage of CD4+, Vβ3+ T cells occurred as rapidly as in
5   mice injected with the recombinant SEA protein (rSEA). The
deletion, however, observed over the next 2 months in mice
injected with PCR$_3$-SEA.S DNA was longer lasting and was more
pronounced than the deletion induced by injection of SEA.S
protein. In addition, injection of as little as 2 μg PCR$_3$-
10   SEA.S DNA also induced deletion of Vβ3+ T cells. Thus,
intramuscular injection of DNA encoding superantigens
represents a potent and non-toxic approach to the deletion
or suppression of potentially harmful (e.g., autoreactive
T cells) T cells.

15   <u>Example 12</u>

This example describes the production of immunogen and
chemokine encoding recombinant molecules.

Recombinant molecules encoding ovalbumin (OVA) were
produced by ligating cDNA encoding OVA into the eukaryotic
20   expression vector PCR$_3$ and is referred to herein as PCR$_3$-
OVA. cDNA encoding murine RANTES, murine macrophage
inflammatory protein-1 alpha (MIP-1α), and macrophage
inflammatory protein-1 beta (MIP-1β) was prepared from RNA
isolated from LPS-stimulated normal murine bone marrow
25   macrophages using methods standard in the art. The cDNA
were ligated into the expression vector PCR$_3$, and are
referred to herein as PCR$_3$-RANTES, PCR$_3$-MIP-1α and PCR$_3$-MIP-

1β.   All plasmid DNA were purified by cesium chloride gradient centrifugation and resuspended at 1.0 mg/ml in sterile PBS.

Example 13

5   This example demonstrates that the co-administration of adjuvant DNA and immunogen DNA stimulates antibody production against the immunogen protein.

Separate groups of 4 CB6 F1 mice per group were injected twice with the following mixtures of DNA: (1)

10   about 100 μg PCR$_3$-OVA + about 100 μg PCR$_3$-MIP-1β; (2) about 100 μg PCR$_3$-OVA + about 50 μg PCR$_3$-SEB (described in Example 1) + PCR$_3$-GM-CSF (described in Example 1); (3) about 100 μg PCR$_3$-OVA + about 100 μg PCR$_3$-RANTES; (4) about 100 μg PCR$_3$- OVA + about 100 μg PCR$_3$-SEB; (5) about 100 μg PCR$_3$-OVA +

15   about 100 μg PCR$_3$-GM-CSF; or (6) about 100 μg PCR$_3$-OVA alone.   Control samples were also prepared which included 6 non-injected, syngeneic mice.   The DNA was diluted to a final concentration of 0.5 mg/ml in sterile phosphate buffered saline (PBS) prior to injection.   The mice were

20   injected intramuscularly, bilaterally in their quadriceps muscles (about 100 μg of DNA per quadricep).

About 20 days after the immunization of step B, serum was collected from each mouse and assayed for antibodies that specifically bind to OVA protein using an OVA-specific

25   enzyme linked immunoassay (ELISA) assay using methods standard in the art.   Briefly, OVA protein was bound to an ELISA plate.   The plates were washed and then incubated in

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the presence of serum. Again the plates were washed and then incubated in the presence of HRP-conjugated anti-mouse IgG antibody. The amount of antibody bound to the OVA was detected on an ELISA reader and are expressed in absorbance

5   units.

The results of the ELISA are shown in Fig. 9 and indicate that co-injection of DNA encoding OVA, with either DNA encoding RANTES or MIP-1β, or SEB and GM-CSF, increases the antibody response to OVA over that observed with OVA

10   alone, OVA plus GM-CSF, OVA plus SEB alone or control samples. Thus, the expression of RANTES, MIP-1β, or SEB and GM-CSF increase the antibody response to OVA when administered as a DNA vaccine.

Example 14

15   This example demonstrates that the co-administration of DNA adjuvant and immunogen DNA results in the production of interferon gamma.

Separate groups of 4 CB6 F1 mice per group were injected twice, intramuscularly (on day 1 and day 21), with

20   the following mixtures of DNA: (1) about 100 μg PCR$_3$-OVA + about 100 μg PCR$_3$-MIP-1β; (2) about 100 μg PCR$_3$-OVA + about 50 μg PCR$_3$-SEB + PCR$_3$-GM-CSF; (3) about 100 μg PCR$_3$-OVA + about 100 μg PCR$_3$-RANTES; (4) about 100 μg PCR$_3$-OVA + about 100 μg PCR$_3$-SEB; (5) about 100 μg PCR$_3$-OVA + about 100 μg

25   PCR$_3$-GM-CSF; or (6) about 100 μg PCR$_3$-OVA alone. Control samples were also prepared as above.

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The mice were sacrificed on day 27. Spleen cells were harvested from each mouse and re-stimulated *in vitro* with irradiated OVA-transfected cells (EG7-OVA) in quadruplicate wells. On day 4 of the re-stimulation with

5 irradiated EG7-OVA cells, supernatants were harvested from the cultures and assayed for interferon gamma activity using an interferon gamma-specific ELISA assay. Results were expressed as units/ml of interferon activity, as determined by comparison with a standard curve generated

10 with recombinant murine interferon-gamma.

The results are shown in Fig. 10 and indicate that RANTES or GM-CSF were effective compounds for inducing interferon-gamma production. Although less, SEB and MIP-1β also induced interferon-gamma production. Additional

15 experiments indicated that none of the adjuvants evaluated in this experiment induced significant quantities of IL-4 release. Together, these data indicate that the immune response induced by an adjuvant of the present invention is primarily a Th1 response, which induces primarily cell-

20 mediated immunity, including macrophage activation, enhanced T cell CTL activity, and increased MHC expression.

Example 15

This example demonstrates that the co-administration of adjuvant DNA and immunogen DNA induce T cell

25 proliferative responses to the immunogen.

Separate group of 4 CB6 F1 mice per group were immunized using the protocol described in Example 14. The

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animals were sacrificed on day 27 and harvested spleen cells re-stimulated using the method described in Example 14. After about 4 days of re-stimulation, 100 µl aliquots of the cells were harvested from each well and pulsed for

5   18 hours with ^{3}H-thymidine. Thymidine incorporation was then quantitated (cpm) as a measure of the proliferative response to OVA expressed by the transfected EG7-OVA cell line.

The results are shown in Fig. 11 and indicate that

10   MIP-1β, RANTES, SEB + GM-CSF, and SEB alone, when co-administered together with OVA DNA, induce a substantial increase in the proliferative response to OVA. Thus, these data provide evidence that DNA encoding chemokines and SAgs are useful for enhancing cell-mediated immune responses and

15   therefore are useful as DNA vaccine adjuvants.

Example 16

This example demonstrates that the co-administration of adjuvant DNA increases CTL responses to the immunogen ovalbumin.

20   Mice were immunized using the protocol described in Example 14. Spleen cells were harvested from the immunized mice 7 days after the last vaccination. The cells were then re-stimulated in vitro for 6 days with irradiated EG7-OVA cells. T cells were then harvested from the re-

25   stimulated population and added in decreasing numbers to ^{51}Cr-labeled EG7-OVA or EL-4 target cells in a standard 4 hour chromium release assay for CTL activity. The percent

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cell lysis was determined Chromium release was then
quantitated (cpm) as a measure of the percent specific cell
lysis of labeled target cells. The higher the % specific
lysis, the more CTL activity exhibited by the T cells.

5   The results are shown in Fig. 12 and indicate that all
of the adjuvant DNAs evaluated induced increased CTL
activity compared to OVA alone. The use of RANTES, GM-CSF
and SEB alone, each were effective in inducing CTL
activity. These data indicate that co-administration of
10   chemokine DNA can enhance CTL-mediated immunity to an
intracellular immunogen, as typified by OVA expressed in a
transfected cell line, indicating that this approach is
useful for vaccines against intracellular pathogens.

        Taken together, the results of Examples 12-16 indicate
15   that all DNA adjuvants tested (GM-CSF, SEB, SEB+GM-CSF,
RANTES and MIP-1β) improved cell mediated immunity against
the immunogen ovalbumin. In particular, the use of either
SEB or GM-CSF alone, as well as the combination of SEB +
GM-CSF were effective at inducing cell mediated immunity.

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SEQUENCE LISTING

The following Sequence Listing is submitted pursuant to 37 CFR §1.821. A copy in computer readable form is also submitted herewith.

5   Applicants assert pursuant to 37 CFR §1.821(f) that the content of the paper and computer readable copies of SEQ ID NO:1 through SEQ ID NO:13 submitted herewith are the same.

(1) GENERAL INFORMATION:

10   (i) APPLICANT: Dow, Steve W.
                       Elmslie, Robyn E.
                       Potter, Terence A.

         (ii) TITLE OF INVENTION: GENE THERAPY FOR EFFECTOR CELL
              REGULATION

15   (iii) NUMBER OF SEQUENCES: 13

         (iv) CORRESPONDENCE ADDRESS:
              (A) ADDRESSEE: Sheridan Ross & McIntosh
              (B) STREET: 1700 Lincoln Street, Suite 3500
20   (C) CITY: Denver
              (D) STATE: Colorado
              (E) COUNTRY: U.S.A.
              (F) ZIP: 80203

          (v) COMPUTER READABLE FORM:
25   (A) MEDIUM TYPE: Floppy disk
              (B) COMPUTER: IBM PC compatible
              (C) OPERATING SYSTEM: PC-DOS/MS-DOS
              (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

         (vi) CURRENT APPLICATION DATA:
30   (A) APPLICATION NUMBER:
              (B) FILING DATE:
              (C) CLASSIFICATION:

       (viii) ATTORNEY/AGENT INFORMATION:
              (A) NAME: Connell, Gary J.
35   (B) REGISTRATION NUMBER: 32,020
              (C) REFERENCE/DOCKET NUMBER: 2879-29-C1-PCT

         (ix) TELECOMMUNICATION INFORMATION:
              (A) TELEPHONE: (303) 863-9700
              (B) TELEFAX: (303) 863-0223

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        (2) INFORMATION FOR SEQ ID NO:1:

              (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 773 base pairs
                    (B) TYPE: nucleic acid
    5   (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

              (ii) MOLECULE TYPE: protein

              (ix) FEATURE:
                    (A) NAME/KEY: CDS
    10   (B) LOCATION: 1..765

              (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

        ATG ACC ATG ATT ACG AAT TTA ATA CGA CTC ACT ATA GGG AAT TCC ATG   48
        Met Thr Met Ile Thr Asn Leu Ile Arg Leu Thr Ile Gly Asn Ser Met
         1   5   10   15

    15 GAG AGT CAA CCA GAT CCT AAA CCA GAT GAG TTG CAC AAA TCG AGT AAA   96
        Glu Ser Gln Pro Asp Pro Lys Pro Asp Glu Leu His Lys Ser Ser Lys
                         20   25   30

        TTC ACT GGT TTG ATG GAA AAT ATG AAA GTT TTG TAT GAT GAT AAT CAT   144
        Phe Thr Gly Leu Met Glu Asn Met Lys Val Leu Tyr Asp Asp Asn His
    20   35   40   45

        GTA TCA GCA ATA AAC GTT AAA TCT ATA GAT CAA TTT CTA TAC TTT GAC   192
        Val Ser Ala Ile Asn Val Lys Ser Ile Asp Gln Phe Leu Tyr Phe Asp
                     50   55   60

        TTA ATA TAT TCT ATT AAG GAC ACT AAG TTA GGG AAT TAT GAT AAT GTT   240
    25 Leu Ile Tyr Ser Ile Lys Asp Thr Lys Leu Gly Asn Tyr Asp Asn Val
         65   70   75   80

        CGA GTC GAA TTT AAA AAC AAA GAT TTA GCT GAT AAA TAC AAA GAT AAA   288
        Arg Val Glu Phe Lys Asn Lys Asp Leu Ala Asp Lys Tyr Lys Asp Lys
                         85   90   95

    30 TAC GTA GAT GTG TTT GGA GCT AAT TAT TAT TAT CAA TGT TAT TTT TCT   336
        Tyr Val Asp Val Phe Gly Ala Asn Tyr Tyr Tyr Gln Cys Tyr Phe Ser
                     100   105   110

        AAA AAA ACG AAT GAT ATT AAT TCG CAT CAA ACT GAC AAA CGA AAA ACT   384
        Lys Lys Thr Asn Asp Ile Asn Ser His Gln Thr Asp Lys Arg Lys Thr
    35   115   120   125

        TGT ATG TAT GGT GGT GTA ACT GAG CAT AAT GGA AAC CAA TTA GAT AAA   432
        Cys Met Tyr Gly Gly Val Thr Glu His Asn Gly Asn Gln Leu Asp Lys
                 130   135   140

        TAT AGA AGT ATT ACT GTT CGG GTA TTT GAA GAT GGT AAA AAT TTA TTA   480
    40 Tyr Arg Ser Ile Thr Val Arg Val Phe Glu Asp Gly Lys Asn Leu Leu
        145   150   155   160

        TCT TTT GAC GTA CAA ACT AAT AAG AAA AAG GTG ACT GCT CAA GAA TTA   528
        Ser Phe Asp Val Gln Thr Asn Lys Lys Lys Val Thr Ala Gln Glu Leu
                     165   170   175

    45 GAT TAC CTA ACT CGT CAC TAT TTG GTG AAA AAT AAA AAA CTC TAT GAA   576
        Asp Tyr Leu Thr Arg His Tyr Leu Val Lys Asn Lys Lys Leu Tyr Glu
                     180   185   190

```
TTT AAC AAC TCG CCT TAT GAA ACG GGA TAT ATT AAA TTT ATA GAA AAT        624
Phe Asn Asn Ser Pro Tyr Glu Thr Gly Tyr Ile Lys Phe Ile Glu Asn
        195                 200                 205
```

```
GAG AAT AGC TTT TGG TAT GAC ATG ATG CCT GCA CCA GGA GAT AAA TTT        672
Glu Asn Ser Phe Trp Tyr Asp Met Met Pro Ala Pro Gly Asp Lys Phe
        210                 215                 220
```

```
GAC CAA TCT AAA TAT TTA ATG ATG TAC AAT GAC AAT AAA ATG GTT GAT        720
Asp Gln Ser Lys Tyr Leu Met Met Tyr Asn Asp Asn Lys Met Val Asp
225                 230                 235                 240
```

```
TCT AAA GAT GTG AAG ATT GAA GTT TAT CTT ACG ACA AAG AAA AAG            765
Ser Lys Asp Val Lys Ile Glu Val Tyr Leu Thr Thr Lys Lys Lys
                245                 250                 255
```

```
TGAAGCTT                                                               773
```

(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 255 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Thr Met Ile Thr Asn Leu Ile Arg Leu Thr Ile Gly Asn Ser Met
 1           5               10              15

Glu Ser Gln Pro Asp Pro Lys Pro Asp Glu Leu His Lys Ser Ser Lys
            20              25              30

Phe Thr Gly Leu Met Glu Asn Met Lys Val Leu Tyr Asp Asp Asn His
            35              40              45

Val Ser Ala Ile Asn Val Lys Ser Ile Asp Gln Phe Leu Tyr Phe Asp
    50              55              60

Leu Ile Tyr Ser Ile Lys Asp Thr Lys Leu Gly Asn Tyr Asp Asn Val
 65              70              75              80

Arg Val Glu Phe Lys Asn Lys Asp Leu Ala Asp Lys Tyr Lys Asp Lys
            85              90              95

Tyr Val Asp Val Phe Gly Ala Asn Tyr Tyr Gln Cys Tyr Phe Ser
            100             105             110

Lys Lys Thr Asn Asp Ile Asn Ser His Gln Thr Asp Lys Arg Lys Thr
            115             120             125

Cys Met Tyr Gly Gly Val Thr Glu His Asn Gly Asn Gln Leu Asp Lys
        130             135             140

Tyr Arg Ser Ile Thr Val Arg Val Phe Glu Asp Gly Lys Asn Leu Leu
145             150             155             160

Ser Phe Asp Val Gln Thr Asn Lys Lys Lys Val Thr Ala Gln Glu Leu
            165             170             175

Asp Tyr Leu Thr Arg His Tyr Leu Val Lys Asn Lys Lys Leu Tyr Glu
            180             185             190
```

```
     Phe Asn Asn Ser Pro Tyr Glu Thr Gly Tyr Ile Lys Phe Ile Glu Asn
             195                 200                 205

     Glu Asn Ser Phe Trp Tyr Asp Met Met Pro Ala Pro Gly Asp Lys Phe
             210                 215                 220

   5 Asp Gln Ser Lys Tyr Leu Met Met Tyr Asn Asp Asn Lys Met Val Asp
     225                 230                 235                 240

     Ser Lys Asp Val Lys Ile Glu Val Tyr Leu Thr Thr Lys Lys Lys
                     245                 250                 255


         (2) INFORMATION FOR SEQ ID NO:3:

  10         (i) SEQUENCE CHARACTERISTICS:
                 (A) LENGTH: 751 base pairs
                 (B) TYPE: nucleic acid
                 (C) STRANDEDNESS: single
                 (D) TOPOLOGY: linear

  15        (ii) MOLECULE TYPE: protein

            (ix) FEATURE:
                 (A) NAME/KEY: CDS
                 (B) LOCATION: 46..744


            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

  20  ATGACCATGA TTACGAATTT AATACGACTC ACTATAGGGA ATTCC ATG GAG AAA          54
                                                       Met Glu Lys
                                                         1

      AGC GAA GAA ATA AAT GAG AAA GAT CTG CGC AAG AAG TCC GAA TTG CAG     102
      Ser Glu Glu Ile Asn Glu Lys Asp Leu Arg Lys Lys Ser Glu Leu Gln
  25       5                  10                  15

      GGA ACA GCC CTA GGC AAT CTT AAA CAA ATC TAT TAT TAC AAT GAA AAA     150
      Gly Thr Ala Leu Gly Asn Leu Lys Gln Ile Tyr Tyr Tyr Asn Glu Lys
          20                  25                  30                  35

      GCG AAG ACT GAG AAT AAA GAG AGT CAC GAT CAA TTT CTG CAG CAT ACT     198
      Ala Lys Thr Glu Asn Lys Glu Ser His Asp Gln Phe Leu Gln His Thr
  30                  40                  45                  50

      ATA TTG TTT AAA GGC TTT TTT ACT GAT CAT TCG TGG TAT AAC GAT TTA     246
      Ile Leu Phe Lys Gly Phe Phe Thr Asp His Ser Trp Tyr Asn Asp Leu
                      55                  60                  65

  35  CTA GTA GAT TTT GAT TCG AAG GAC ATC GTT GAT AAA TAT AAA GGG AAG     294
      Leu Val Asp Phe Asp Ser Lys Asp Ile Val Asp Lys Tyr Lys Gly Lys
                      70                  75                  80

      AAG GTC GAC TTG TAT GGT GCT TAT TAT GGG TAC CAA TGT GCT GGT GGT     342
      Lys Val Asp Leu Tyr Gly Ala Tyr Tyr Gly Tyr Gln Cys Ala Gly Gly
  40       85                  90                  95

      ACA CCA AAC AAA ACA GCA TGC ATG TAT GGT GGG GTA ACC TTA CAT GAC     390
      Thr Pro Asn Lys Thr Ala Cys Met Tyr Gly Gly Val Thr Leu His Asp
      100                 105                 110                 115

      AAT AAT CGA TTG ACC GAA GAG AAA AAG GTC CCG ATC AAT TTA TGG CTA     438
  45  Asn Asn Arg Leu Thr Glu Glu Lys Lys Val Pro Ile Asn Leu Trp Leu
                      120                 125                 130
```

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```
GAC GGT AAA CAA AAT ACA GTA CCT CTA GAA ACG GTT AAA ACG AAT AAG      486
Asp Gly Lys Gln Asn Thr Val Pro Leu Glu Thr Val Lys Thr Asn Lys
            135                 140                 145

AAA AAT GTA ACT GTC CAA GAG CTG GAT CTT CAA GCG CGC CGA TAC CTA      534
Lys Asn Val Thr Val Gln Glu Leu Asp Leu Gln Ala Arg Arg Tyr Leu
            150                 155                 160

CAG GAA AAA TAT AAT TTG TAC AAC TCT GAC GTC TTT GAT GGG AAG GTT      582
Gln Glu Lys Tyr Asn Leu Tyr Asn Ser Asp Val Phe Asp Gly Lys Val
            165                 170                 175

CAG AGA GGC CTA ATC GTG TTT CAT ACT TCT ACA GAA CCT TCG GTT AAC      630
Gln Arg Gly Leu Ile Val Phe His Thr Ser Thr Glu Pro Ser Val Asn
180                 185                 190                 195

TAC GAT TTA TTT GGA GCT CAA GGA CAG TAT TCA AAT ACA CTC TTA AGA      678
Tyr Asp Leu Phe Gly Ala Gln Gly Gln Tyr Ser Asn Thr Leu Leu Arg
                    200                 205                 210

ATA TAT CGC GAC AAC AAG ACG ATT AAC TCT GAA AAC ATG CAC ATT GAT      726
Ile Tyr Arg Asp Asn Lys Thr Ile Asn Ser Glu Asn Met His Ile Asp
            215                 220                 225

ATC TAT TTA TAT ACA AGT TAAGCTT                                      751
Ile Tyr Leu Tyr Thr Ser
            230
```

(2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 233 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Glu Lys Ser Glu Glu Ile Asn Glu Lys Asp Leu Arg Lys Lys Ser
 1               5                  10                  15

Glu Leu Gln Gly Thr Ala Leu Gly Asn Leu Lys Gln Ile Tyr Tyr Tyr
            20                  25                  30

Asn Glu Lys Ala Lys Thr Glu Asn Lys Glu Ser His Asp Gln Phe Leu
        35                  40                  45

Gln His Thr Ile Leu Phe Lys Gly Phe Phe Thr Asp His Ser Trp Tyr
        50                  55                  60

Asn Asp Leu Leu Val Asp Phe Asp Ser Lys Asp Ile Val Asp Lys Tyr
65                  70                  75                  80

Lys Gly Lys Lys Val Asp Leu Tyr Gly Ala Tyr Tyr Gly Tyr Gln Cys
                85                  90                  95

Ala Gly Gly Thr Pro Asn Lys Thr Ala Cys Met Tyr Gly Gly Val Thr
            100                 105                 110

Leu His Asp Asn Asn Arg Leu Thr Glu Glu Lys Lys Val Pro Ile Asn
        115                 120                 125

Leu Trp Leu Asp Gly Lys Gln Asn Thr Val Pro Leu Glu Thr Val Lys
        130                 135                 140
```

```
    Thr Asn Lys Lys Asn Val Thr Val Gln Glu Leu Asp Leu Gln Ala Arg
    145             150             155             160

    Arg Tyr Leu Gln Glu Lys Tyr Asn Leu Tyr Asn Ser Asp Val Phe Asp
                165             170             175

5   Gly Lys Val Gln Arg Gly Leu Ile Val Phe His Thr Ser Thr Glu Pro
                180             185             190

    Ser Val Asn Tyr Asp Leu Phe Gly Ala Gln Gly Gln Tyr Ser Asn Thr
                195             200             205

10  Leu Leu Arg Ile Tyr Arg Asp Asn Lys Thr Ile Asn Ser Glu Asn Met
        210             215             220

    His Ile Asp Ile Tyr Leu Tyr Thr Ser
    225             230
```

(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 582 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 1..582

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
ATG ACA AAC GAT AAT ATA AAG GAT TTG CTA GAC TGG TAT AGT AGT GGG      48
Met Thr Asn Asp Asn Ile Lys Asp Leu Leu Asp Trp Tyr Ser Ser Gly
 1               5               10              15

TCT GAC ACT TTT ACA AAT AGT GAA GTT TTA GAT AAT TCC TTA GGA TCT      96
Ser Asp Thr Phe Thr Asn Ser Glu Val Leu Asp Asn Ser Leu Gly Ser
            20              25              30

ATG CGT ATA AAA AAC ACA GAT GGC AGC ATC AGC CTT ATA ATT TTT CCG     144
Met Arg Ile Lys Asn Thr Asp Gly Ser Ile Ser Leu Ile Ile Phe Pro
            35              40              45

AGT CCT TAT TAT AGC CCT GCT TTT ACA AAA GGG GAA AAA GTT GAC TTA     192
Ser Pro Tyr Tyr Ser Pro Ala Phe Thr Lys Gly Glu Lys Val Asp Leu
    50              55              60

AAC ACA AAA AGA ACT AAA AAA AGC CAA CAT ACT AGC GAA GGA ACT TAT     240
Asn Thr Lys Arg Thr Lys Lys Ser Gln His Thr Ser Glu Gly Thr Tyr
 65              70              75              80

ATC CAT TTC CAA ATA AGT GGC GTT ACA AAT ACT GAA AAA TTA CCT ACT     288
Ile His Phe Gln Ile Ser Gly Val Thr Asn Thr Glu Lys Leu Pro Thr
                85              90              95

CCA ATA GAA CTA CCT TTA AAA GTT AAG GTT CAT GGT AAA GAT AGC CCC     336
Pro Ile Glu Leu Pro Leu Lys Val Lys Val His Gly Lys Asp Ser Pro
            100             105             110

TTA AAG TAT TGG CCA AAG TTC GAT AAA AAA CAA TTA GCT ATA TCA ACT     384
Leu Lys Tyr Trp Pro Lys Phe Asp Lys Lys Gln Leu Ala Ile Ser Thr
            115             120             125
```

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```
      TTA GAC TTT GAA ATT CGT CAT CAG CTA ACT CAA ATA CAT GGA TTA TAT    432
      Leu Asp Phe Glu Ile Arg His Gln Leu Thr Gln Ile His Gly Leu Tyr
          130                 135                 140

  5   CGT TCA AGC GAT AAA ACG GGT GGT TAT TGG AAA ATA ACA ATG AAT GAC    480
      Arg Ser Ser Asp Lys Thr Gly Gly Tyr Trp Lys Ile Thr Met Asn Asp
      145                 150                 155                 160

      GGA TCC ACA TAT CAA AGT GAT TTA TCT AAA AAG TTT GAA TAC AAT ACT    528
      Gly Ser Thr Tyr Gln Ser Asp Leu Ser Lys Lys Phe Glu Tyr Asn Thr
                          165                 170                 175

 10   GAA AAA CCA CCT ATA AAT ATT GAT GAA ATA AAA ACT ATA GAA GCA GAA    576
      Glu Lys Pro Pro Ile Asn Ile Asp Glu Ile Lys Thr Ile Glu Ala Glu
                      180                 185                 190

      ATT AAT                                                            582
      Ile Asn
 15
```

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 194 amino acids
        (B) TYPE: amino acid
 20   (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
      Met Thr Asn Asp Asn Ile Lys Asp Leu Leu Asp Trp Tyr Ser Ser Gly
       1               5                  10                  15

 25   Ser Asp Thr Phe Thr Asn Ser Glu Val Leu Asp Asn Ser Leu Gly Ser
                      20                  25                  30

      Met Arg Ile Lys Asn Thr Asp Gly Ser Ile Ser Leu Ile Ile Phe Pro
              35                  40                  45

      Ser Pro Tyr Tyr Ser Pro Ala Phe Thr Lys Gly Glu Lys Val Asp Leu
 30       50                  55                  60

      Asn Thr Lys Arg Thr Lys Lys Ser Gln His Thr Ser Glu Gly Thr Tyr
       65                  70                  75                  80

      Ile His Phe Gln Ile Ser Gly Val Thr Asn Thr Glu Lys Leu Pro Thr
                      85                  90                  95

 35   Pro Ile Glu Leu Pro Leu Lys Val Lys Val His Gly Lys Asp Ser Pro
                      100                 105                 110

      Leu Lys Tyr Trp Pro Lys Phe Asp Lys Lys Gln Leu Ala Ile Ser Thr
                  115                 120                 125

      Leu Asp Phe Glu Ile Arg His Gln Leu Thr Gln Ile His Gly Leu Tyr
 40       130                 135                 140

      Arg Ser Ser Asp Lys Thr Gly Gly Tyr Trp Lys Ile Thr Met Asn Asp
      145                 150                 155                 160

      Gly Ser Thr Tyr Gln Ser Asp Leu Ser Lys Lys Phe Glu Tyr Asn Thr
                          165                 170                 175

 45   Glu Lys Pro Pro Ile Asn Ile Asp Glu Ile Lys Thr Ile Glu Ala Glu
                      180                 185                 190

      Ile Asn
```

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(2) INFORMATION FOR SEQ ID NO:7:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA (genomic)

        (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 1..25
            (D) OTHER INFORMATION: /label= primer

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGGAATTCCA TGGAGAGTCA ACCAG   25

(2) INFORMATION FOR SEQ ID NO:8:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 23 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA (genomic)

        (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 1..23
            (D) OTHER INFORMATION: /label= primer

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCGGATCCTC ACTTTTTCTT TGT   23

(2) INFORMATION FOR SEQ ID NO:9:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 22 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA (genomic)

        (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 1..22
            (D) OTHER INFORMATION: /label= primer

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGGAATTCCA TGGAGAAAAG CG   22

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(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 25 base pairs
       (B) TYPE: nucleic acid
5   (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
       (A) NAME/KEY: misc_feature
10   (B) LOCATION: 1..25
       (D) OTHER INFORMATION: /label= primer

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCAAGCTTAA CTTGTATATA AATAG   25

(2) INFORMATION FOR SEQ ID NO:11:

15   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 51 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

20   (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
       (A) NAME/KEY: misc_feature
       (B) LOCATION: 1..51

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

25 CGGGGTACCC CGAAGGAGGA AAAAAAAATG TCTACAAACG ATAATATAAA G   51

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 42 base pairs
       (B) TYPE: nucleic acid
30   (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
       (A) NAME/KEY: misc_feature
35   (B) LOCATION: 1..42
       (D) OTHER INFORMATION: /label= primer

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TGCTCTAGAG CATTAATTAA TTTCTGCTTC TATAGTTTTT AT   42

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(2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 279 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ACCATGAAGA TCTCTGCAGC TGCCCTCACC ATCATCCTCA CTGCAGCCGC CCTCTGGGCG   60

CCCGCGCCTG CCTCACCATA TGGCTCGGAC ACCACTCCCT GCTGCTTTGC CTACCTCTCC   120

CTCGCGCTGC CTCGTGCCCA CGTCAAGGAG TATTTCTACA CCAGCAGCAA GTGCTCCAAT   180

CTTGCAGTCG TGTTTGTCAC TCGAAGGAAC CGCCAAGTGT GTGCCAACCC AGAGAAGAAG   240

TGGGTTCAAG AATACATCAA CTATTTGGAG ATGAGCTAG   279

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims:

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<u>What is claimed</u>:

1.   A therapeutic composition comprising: (a) an isolated nucleic acid molecule encoding a superantigen; and (b) an isolated nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine and mixtures thereof, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

2.   The therapeutic composition of Claim 1, wherein said superantigen is selected from the group consisting of staphylococcal enterotoxins, retroviral antigens, streptococcal antigens, mycoplasma antigens, mycobacteria antigens, viral antigens and protozoan antigens.

3.   The therapeutic composition of Claim 1, wherein said superantigen comprises staphylococcal enterotoxins.

4.   The therapeutic composition of Claim 1, wherein said superantigen is selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

5.   The therapeutic composition of Claim 1, wherein said superantigen is derived from a virus selected from the group consisting of mouse mammary tumor virus, rabies virus and herpes virus.

6.   The therapeutic composition of Claim 1, wherein said cytokine is selected from the group consisting of hematopoietic growth factors, interleukins, interferons,

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immunoglobulin superfamily molecules, tumor necrosis factor family molecules and chemokines.

7. The therapeutic composition of Claim 1, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, and interleukin-15.

8. The therapeutic composition of Claim 1, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor and tumor necrosis factor α.

9. The therapeutic composition of Claim 1, wherein said cytokine comprises granulocyte macrophage colony stimulating factor.

10. The therapeutic composition of Claim 1, wherein said chemokine is selected from the group consisting of C5a, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB$_4$R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

11. The therapeutic composition of Claim 1, wherein said chemokine is selected from the group consisting of IL-8, MIP1α, MIP1β, MCP-1, MCP-3, RANTES and NAP-2.

12. The therapeutic composition of Claim 1, wherein said chemokine is selected from the group consisting of IL-8, Rantes, MIP1α and MIP1β.

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13. The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences capable of being expressed in a mammalian cell.

14. The therapeutic composition of Claim 1, wherein said transcription control sequences are selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR sequences, SV-40 control sequences and β-actin control sequences.

15. The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecule encoding a superantigen comprises a recombinant molecule selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST.

16. The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecule encoding a cytokine comprises PCR_3-GM_3.

17. The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecule encoding a chemokine is selected from the group consisting of PCR_3-RANTES, PCR_3-MIP1α and PCR_3-MIP1β.

18. The therapeutic composition of Claim 1, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-

containing substrate, an oil, an ester, a glycol, a virus and a metal particle.

19.   The therapeutic composition of Claim 18, wherein said pharmaceutically acceptable carrier is selected from the group consisting of liposomes and an aqueous physiologically balanced solution.

20.   The therapeutic composition of Claim 1, wherein said therapeutic composition further comprises an immunogen selected from the group consisting of a pathogen, an allergen, tumor antigens and self-antigens.

21.   The therapeutic composition of Claim 1, wherein said therapeutic composition further comprises an immunogen comprising a peptide derived from *Mycobacterium tuberculosis*.

22.   A recombinant molecule comprising: (a) a first isolated nucleic acid molecule encoding a superantigen; and (b) a second isolated nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

23.   The molecule of Claim 22, wherein said recombinant molecule contains a transcription control sequence selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR control

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sequences, SV-40 control sequences and β-actin control sequences.

24. The molecule of Claim 22, wherein said recombinant molecule is dicistronic and comprises an IRES.

25. The molecule of Claim 22, wherein said first nucleic acid molecule and said second nucleic acid molecule are separated by an IRES.

26. The molecule of Claim 22, wherein said first nucleic acid molecule encodes a superantigen selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

27. The molecule of Claim 22, wherein said second nucleic acid molecule encodes a protein selected from the group consisting of macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, interleukin-15, C5a, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB_4R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

28. A recombinant molecule comprising: (a) a first isolated nucleic acid molecule encoding a first superantigen; and (b) a second isolated nucleic acid molecule encoding a second superantigen, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

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29. The molecule of Claim 28, wherein said recombinant molecule contains a transcription control sequence selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR control sequences, SV-40 control sequences and β-actin control sequences.

30. The molecule of Claim 28, wherein said recombinant molecule is dicistronic and comprises an IRES.

31. The molecule of Claim 28, wherein said first nucleic acid molecule and said second nucleic acid molecule are separated by an IRES.

32. The molecule of Claim 28, wherein said first nucleic acid molecule encodes a superantigen selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

33. A therapeutic composition comprising a delivery vehicle carrying: (a) a first isolated nucleic acid molecule encoding a superantigen; and (b) a second isolated nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine and mixtures thereof, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

34. The therapeutic composition of Claim 33, wherein said delivery vehicle comprises a liposome.

35. The therapeutic composition of Claim 33, wherein said first nucleic acid molecule encodes a superantigen

selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

36. The therapeutic composition of Claim 33, wherein said second nucleic acid molecule encodes a protein selected from the group consisting of macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, interleukin-15, C5a, IL-8, $MIP1\alpha$, $MIP1\beta$, MCP-1, MCP-3, PAFR, FMLPR, LTB_4R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

37. The therapeutic composition of Claim 33, wherein said delivery vehicle further carries a nucleic acid molecule encoding an immunogen.

38. The therapeutic composition of Claim 33, wherein said delivery vehicle further carries a peptide derived from *Mycobacterium tuberculosis*.

39. A therapeutic composition comprising a delivery vehicle carrying an isolated nucleic acid molecule encoding a superantigen, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

40. The therapeutic composition of Claim 39, wherein said delivery vehicle comprises a liposome.

41. The therapeutic composition of Claim 39, wherein said nucleic acid molecule encodes a superantigen selected

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from the group consisting of SEA, SEB, SEC$_1$, SEC$_2$, SEC$_3$, SED, SEE and TSST.

42. The therapeutic composition of Claim 39, wherein said delivery vehicle further carries a nucleic acid molecule encoding a protein selected from the group consisting of a cytokine, a chemokine and an immunogen.

43. The therapeutic composition of Claim 39, wherein said delivery vehicle further carries a nucleic acid molecule encoding a protein selected from the group consisting of macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, interleukin-15, C5a, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB$_4$R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

44. The therapeutic composition of Claim 39, wherein said delivery vehicle further carries a peptide derived from *Mycobacterium tuberculosis*.

45. An adjuvant composition, comprising an immunogen and an isolated nucleic acid molecule encoding a superantigen, wherein said isolated nucleic acid molecule is operatively linked to one or more transcription control sequences.

46. The adjuvant composition of Claim 45, wherein said adjuvant composition further comprises a nucleic acid

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molecule encoding a protein selected from the group
consisting of a cytokine, a chemokine and mixtures thereof.

47. The adjuvant composition of Claim 45, wherein
said immunogen comprises a compound selected from the group
5 consisting of a nucleic acid molecule and a peptide.

48. The adjuvant composition of Claim 45, wherein
said adjuvant composition comprises up to about 50% of a
nucleic acid molecule encoding an immunogen and up to about
50% of a nucleic acid molecule encoding a superantigen.

10   49. The adjuvant composition of Claim 45, wherein
said adjuvant composition comprises up to about 66% of a
nucleic acid molecule encoding an immunogen and up to about
33% of a nucleic acid molecule encoding a superantigen.

50. The adjuvant composition of Claim 45, wherein
15 said adjuvant composition comprises up to about 50% of a
nucleic acid molecule encoding an immunogen, up to about
25% of a nucleic acid molecule encoding a superantigen and
up to about 25% of a nucleic acid molecule selected from
the group consisting of a nucleic acid molecule encoding a
20 cytokine, a nucleic acid molecule encoding a chemokine and
mixtures thereof.

51. The adjuvant composition of Claim 45, wherein
said nucleic acid molecules comprise naked DNA.

52. The adjuvant composition of Claim 45, wherein
25 said composition further comprises a peptide derived from
Mycobacterium tuberculosis.

53.   A method for increasing effector cell immunity in
an animal, said method comprising administering to an
animal an effective amount of an adjuvant composition
comprising an immunogen and an isolated nucleic acid
5   molecule encoding a superantigen.

54.   The method of Claim 53, wherein said adjuvant
composition further comprises a nucleic acid molecule
encoding a protein selected from the group consisting of a
cytokine, a chemokine and mixtures thereof.

10   55.   The method of Claim 53, wherein said adjuvant
composition further comprises a pharmaceutically acceptable
carrier.

56.   The method of Claim 53, wherein said step of
administering comprises injecting said adjuvant composition
15   by a route selected from the group consisting of
intravenous,   intraperitoneally,   intramuscularly,
intraarterially and directly into a specific tissue site.

57.   The method of Claim 53, wherein said animal is a
mammal.

20   58.   The method of Claim 53, wherein said animal is
selected from the from the group consisting of humans,
horses, dogs, cats and cattle.

59.   A method to treat an animal with cancer, said
method comprising administering to an animal an effective
25   amount of a therapeutic composition comprising: (a) an
isolated nucleic acid molecule encoding a superantigen; and
(b) an isolated nucleic acid molecule selected from the

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group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine, and mixtures thereof, wherein said nucleic acid molecules are operatively linked to one or more transcription control

5   sequences.

60. The method of Claim 59, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-

10 containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and a metal particle.

61. The method of Claim 59, wherein said pharmaceutically acceptable carrier is selected from the group consisting of liposomes, micelles, cells and cellular

15 membranes.

62. The method of Claim 59, wherein said pharmaceutically acceptable carrier comprises a liposome.

63. The method of Claim 59, wherein said pharmaceutically acceptable carrier comprises a liposome

20 that includes a compound capable of specifically targeting said liposome to a tumor cell.

64. The method of Claim 63, wherein said compound is a tumor cell ligand.

65. The method of Claim 59, wherein said therapeutic

25 composition is targeted to the site of a cancer in said animal by administering said therapeutic composition locally within the area of a cancer cell.

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66. The method of Claim 59, wherein said cancer is selected from the group consisting of melanomas, squamous cell carcinoma, breast cancers, head and neck carcinomas, thyroid carcinomas, soft tissue sarcomas, bone sarcomas,

5   testicular cancers, prostatic cancers, ovarian cancers, bladder cancers, skin cancers, brain cancers, angiosarcomas, hemangiosarcomas, mast cell tumors, primary hepatic cancers, lung cancers, pancreatic cancers, gastrointestinal cancers, renal cell carcinomas,

10   hematopoietic neoplasias, leukemias and lymphomas.

67. A method to treat an animal with cancer, said method comprising introducing into a tumor cell *in vivo* an effective amount of a therapeutic composition comprising an isolated nucleic acid molecule encoding a superantigen,

15   wherein said nucleic acid molecules are operatively linked to one or more transcription control sequences.

68. The method of Claim 67, wherein said isolated nucleic acid molecule encoding a superantigen comprises a recombinant molecule selected from the group consisting of

20   PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST.

69. The method of Claim 67, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier comprising a liposome.

70. The method of Claim 67, wherein said therapeutic

25   composition further comprises a pharmaceutically acceptable carrier comprising a liposome that includes a compound

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capable of specifically targeting said liposome to a tumor cell.

71.   The method of Claim 70, wherein said compound is a tumor cell ligand.

5   72.   The method of Claim 67, wherein said therapeutic composition is targeted to the site of a cancer in said animal by administering said therapeutic composition locally within the area of a cancer cell.

73.   The method of Claim 67, wherein said therapeutic

10   composition is administered to said animal at a site comprising a lymph node.

74.   A method to treat an animal with cancer, said method comprising introducing into a non-tumor cell an effective amount of a therapeutic composition comprising an

15   isolated nucleic acid molecule encoding a superantigen, wherein said nucleic acid molecules are operatively linked to one or more transcription control sequences.

75.   The method of Claim 74, wherein said step of administration is performed *in vivo*.

20   76.   The method of Claim 74, wherein said isolated nucleic acid molecule encoding a superantigen comprises a recombinant molecule selected from the group consisting of PCR$_3$-SEB, PCR$_3$-SEA, PCR$_3$-SEB.S, PCR$_3$-SEA.S and PCR$_3$-TSST.

77.   The method of Claim 74, wherein said therapeutic

25   composition comprises a pharmaceutically acceptable carrier comprising a liposome.

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78.   The method of Claim 74, wherein said therapeutic composition is targeted to the site of a cancer in said animal by administering said therapeutic composition locally within the area of a cancer cell.

5   79.   The method of Claim 74, wherein said therapeutic composition is administered to said animal at a site comprising a lymph node.

80.   A method to treat an animal with cancer, said method comprising administering to an animal an effective

10   amount of a therapeutic composition comprising: (a) an isolated nucleic acid molecule encoding a superantigen; and (b) an isolated nucleic acid molecule encoding a cytokine, wherein said nucleic acid molecules are operatively linked to one or more transcription control sequences, and wherein

15   said therapeutic composition is targeted to the site of a cancer in said animal to treat said cancer.

81.   The method of Claim 80, wherein said superantigen is selected from the group consisting of staphylococcal enterotoxins, retroviral antigens, streptococcal antigens,

20   mycoplasma antigens, mycobacteria antigens, viral antigens and protozoan antigens.

82.   The method of Claim 80, wherein said superantigen comprises staphylococcal enterotoxins.

83.   The method of Claim 80, wherein said superantigen

25   is selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

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84. The method of Claim 80, wherein said superantigen is derived from a virus selected from the group consisting of mouse mammary tumor virus, rabies virus and herpes virus.

5   85. The method of Claim 80, wherein said cytokine is selected from the group consisting of hematopoietic growth factors, interleukins, interferons, immunoglobulin superfamily molecules, tumor necrosis factor family molecules and chemokines.

10   86. The method of Claim 80, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-6 and interleukin-12.

15   87. The method of Claim 80, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor and tumor necrosis factor α.

       88. The method of Claim 80, wherein said cytokine

20 comprises granulocyte macrophage colony stimulating factor.

       89. The method of Claim 80, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences capable of being expressed in a mammalian cell.

25   90. The method of Claim 80, wherein said transcription control sequences are selected from the group consisting of RSV control sequences, CMV control sequences,

retroviral LTR sequences, SV-40 control sequences and β-actin control sequences.

91. The method of Claim 80, wherein said isolated nucleic acid molecule comprises a recombinant molecule
5   selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST and PCR_3-GM_3.

92. The method of Claim 80, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier selected from the group consisting of an aqueous
10 physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and a metal particle.

93. The method of Claim 92, wherein said pharmaceutically acceptable carrier is selected from the
15 group consisting of liposomes, micelles, cells and cellular membranes.

94. The method of Claim 92, wherein said pharmaceutically acceptable carrier comprises a liposome.

95. The method of Claim 92, wherein said
20 pharmaceutically acceptable carrier comprises a liposome that includes a compound capable of specifically targeting said liposome to a tumor cell.

96. The method of Claim 95, wherein said compound is a tumor cell ligand.

25   97. The method of Claim 80, wherein said therapeutic composition is targeted to the site of a cancer in said

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animal by administering said therapeutic composition locally within the area of a cancer cell.

98. The method of Claim 80, wherein said cancer is selected from the group consisting of melanomas, squamous cell carcinoma, breast cancers, head and neck carcinomas, thyroid carcinomas, soft tissue sarcomas, bone sarcomas, testicular cancers, prostatic cancers, ovarian cancers, bladder cancers, skin cancers, brain cancers, angiosarcomas, hemangiosarcomas, mast cell tumors, primary hepatic cancers, lung cancers, pancreatic cancers, gastrointestinal cancers, renal cell carcinomas, hematopoietic neoplasias, leukemias and lymphomas.

99. The method of Claim 80, wherein said cancer is selected from the group consisting of melanomas, lung cancers, thyroid carcinomas, breast cancers, renal cell carcinomas, squamous cell carcinomas, brain tumors and skin cancers.

100. The method of Claim 80, wherein said animal is selected from the group consisting of mammals and birds.

101. The method of Claim 80, wherein said animal is selected from the from the group consisting of humans, house pets, economic produce animals and zoo animals.

102. The method of Claim 80, wherein said animal is selected from the from the group consisting of humans, dogs, cats, sheep, cattle, horses and pigs.

103. A therapeutic composition comprising an isolated nucleic acid molecule encoding a superantigen and an

isolated nucleic acid molecule encoding a cytokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

104. The composition of Claim 103, wherein said
5   therapeutic composition further comprises a pharmaceutically acceptable carrier.

105. The composition of Claim 104, wherein said pharmaceutically acceptable carrier is selected from the group consisting of an aqueous physiologically balanced
10 solution, an artificial lipid-containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and metal particles.

106. The composition of Claim 104, wherein said pharmaceutically acceptable carrier comprises a delivery
15 vehicle capable of delivering said nucleic acid molecules to a targeted site in an animal.

107. The composition of Claim 106, wherein said delivery vehicle is selected from the group consisting of liposomes, micelles, cells and cellular membranes.

20 108. The composition of Claim 106, wherein said delivery vehicle comprises a liposome.

109. The composition of Claim 106, wherein said delivery vehicle comprises a tumor cell ligand.

110. The composition of Claim 103, wherein said
25 superantigen is selected from the group consisting of staphylococcal enterotoxins, retroviral antigens,

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streptococcal antigens, mycoplasma antigens, mycobacteria antigens, virus antigens and protozoan antigens.

111. The composition of Claim 103, wherein said superantigen comprises staphylococcal enterotoxins.

5   112. The composition of Claim 103, wherein said superantigen is selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

113. The composition of Claim 103, wherein said superantigen is derived from a virus selected from the

10   group consisting of mouse mammary tumor virus, rabies virus and herpes virus.

114. The composition of Claim 103, wherein said cytokine is selected from the group consisting of hematopoietic growth factors, interleukins, interferons,

15   immunoglobulin superfamily molecules, tumor necrosis factor family molecules and chemokines.

115. The composition of Claim 103, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor,

20   macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-6 and interleukin-12.

116. The composition of Claim 103, wherein said cytokine is granulocyte macrophage colony stimulating factor and tumor necrosis factor α.

25   117. The composition of Claim 103, wherein said isolated nucleic acid molecules are operatively linked to

one or more transcription control sequences capable of
being expressed in a mammalian cell.

118. The composition of Claim 103, wherein said
transcription control sequences are selected from the group
5   consisting of RSV control sequences, CMV control sequences,
retroviral LTR control sequences, SV-40 control sequences
and β-actin control sequences.

119. The composition of Claim 103, wherein said
nucleic acid molecule comprises a recombinant molecule
10   selected from the group consisting of PCR_3-SEB, PCR_3-SEA,
PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST and PCR_3-GM_3.

120. The composition of Claim 103, wherein said
therapeutic composition is useful for treating a cancer
selected from the group consisting of melanomas, squamous
15   cell carcinoma, breast cancers, head and neck carcinomas,
thyroid carcinomas, soft tissue sarcomas, bone sarcomas,
testicular cancers, prostatic cancers, ovarian cancers,
bladder   cancers,   skin   cancers,   brain   cancers,
angiosarcomas, hemangiosarcomas, mast cell tumors, primary
20   hepatic   cancers,   lung   cancers,   pancreatic   cancers,
gastrointestinal   cancers,   renal   cell   carcinomas,
hematopoietic neoplasias, leukemias and lymphomas.

121. The composition of Claim 103, wherein said
therapeutic composition is useful for treating a cancer
25   selected from the group consisting of melanomas, lung
cancers, thyroid carcinomas, breast cancers, renal cell

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carcinomas, squamous cell carcinomas, brain tumors and skin cancers.

122. A recombinant molecule comprising an isolated nucleic acid molecule encoding a superantigen and an isolated nucleic acid molecule encoding a cytokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

123. The molecule of Claim 122, wherein said recombinant molecule is capable being expressed in a mammalian cell.

124. The molecule of Claim 122, wherein said recombinant molecule contains a transcription control sequence selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR control sequences, SV-40 control sequences and β-actin control sequences.

125. The molecule of Claim 122, wherein said superantigen-encoding nucleic acid molecule encodes a toxin selected from the group consisting of staphylococcal enterotoxins, retroviral antigens, streptococcal antigens, mycoplasma antigens, mycobacteria antigens, virus antigens and protozoan antigens.

126. The molecule of Claim 122, wherein said superantigen-encoding nucleic acid molecule encodes a toxin selected from the group consisting of a SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST gene.

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127. The molecule of Claim 122, wherein said superantigen-encoding nucleic acid molecule lacks a bacterial leader sequence.

128. The molecule of Claim 122, wherein said recombinant molecule is selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST and PCR_3-GM_3.

129. A therapeutic composition comprising a delivery vehicle carrying an isolated nucleic acid molecule encoding a superantigen and an isolated nucleic acid molecule encoding a cytokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

130. The composition of Claim 129, wherein said delivery vehicle is selected from the group consisting of a liposome, a micelle, a cell and a cellular membrane.

131. The composition of Claim 129, wherein said delivery vehicle comprises a liposome.

132. The composition of Claim 129, wherein said delivery vehicle comprises DOTMA and DOPE.

133. The composition of Claim 132, wherein said compound is a tumor cell ligand.

134. A method for increasing effector cell immunity in an animal, said method comprising administering to an animal an effective amount of a therapeutic composition comprising:

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a)   an isolated nucleic acid molecule encoding a superantigen; and

b)   an isolated nucleic acid molecule encoding a cytokine,

5   wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences, and wherein said therapeutic composition is targeted to a site in said animal that contains an abnormal cell.

10   135. The method of Claim 134, wherein said abnormal cell is selected from the group consisting of a cancer cell, a cell infected with an infectious agent and a non-cancerous cell having abnormal proliferative growth.

136. The method of Claim 134, wherein said abnormal

15   cell is a cancer cell.

137. The method of Claim 134, wherein said site is a tumor.

138. A method to suppress T cell activity in an animal, said method comprising administering to an animal

20   an effective amount of a therapeutic composition comprising:

a)   a naked isolated nucleic acid molecule encoding a superantigen; and

b)   a pharmaceutically acceptable carrier,

25   wherein said isolated nucleic acid molecule is operatively linked to a traynscription control sequence, and wherein said therapeutic composition is targeted to a

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site in said animal that contains excessive T cell activity.

139. The method of Claim 138, wherein said composition is capable of suppressing said T cell activity for at least
5   about 6 weeks in said animal.

140. The method of Claim 138, wherein said composition is capable of suppressing said T cell activity for at least about 8 weeks in said animal.

141. The method of Claim 138, wherein said composition
10 is capable of suppressing said T cell activity for about 10 weeks in said animal.

142. The method of Claim 138, wherein said carrier comprises an aqueous physiologically balanced solution.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US96/07432

A. CLASSIFICATION OF SUBJECT MATTER

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US CL : 514/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, BIOSIS, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95/00178 A1 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 05 January 1995, see entire document.	1-142
Y	DONNELLY et al. Protective Efficacy of Intramuscular Immunization with Naked DNA. Ann. N.Y. Acad. Sci. 1995, Vol.772, pages 40-46, see entire document.	1-142
Y	BLACKMAN et al. In Vivo Effects of Superantigens. Life Sciences. 1995, Vol.57, No.19, pages 1717-1735, see entire document.	1-142
Y	MIETHKE et al. Superantigen Mediated Shock: A Cytokine Release Syndrome. Immunobiol. 1993, Vol.189, pages 270-284, see entire document.	1-142

☒ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 JULY 1996	23 AUG 1996

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages.	Relevant to claim No.
Y	MIETHKE et al. Superantigens: The Paradox of T-Cell Activation versus Inactivation. Int. Arch. Allergy Immunol. 1995, Vol.106, pages 3-7, see entire document.	1-142
Y	LIU et al. Overview of DNA Vaccines. Ann. N.Y. Acad. Sci. 1995, Vol.772, pages 15-20, see entire document.	1-142

PCT

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(54) Title: GENE THERAPY FOR EFFECTOR CELL REGULATION

(57) Abstract

The present invention provides a nucleic acid-based therapeutic composition to treat an animal with disease by controlling the activity of effector cells, including T cells, macrophages, monocytes and/or natural killer cells, in the animal. Therapeutic compositions of the present invention include superantigen-encoding nucleic acid molecules, either in the presence or absence of a cytokine-encoding nucleic acid molecule and/or chemokine-encoding nucleic acid molecules, depending upon the disease being treated. The present invention also relates to an adjuvant for use with nucleic acid-based vaccines. Adjuvant compositions of the present invention include an immunogen combined with superantigen-encoding nucleic acid molecules, either in the presence or absence of a cytokine-encoding nucleic acid molecule and/or chemokine-encoding nucleic acid molecules.

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GENE THERAPY FOR EFFECTOR CELL REGULATION

FIELD OF THE INVENTION

The present invention relates to a product and process for regulating T cell activity by providing a superantigen gene, in the presence or absence of a cytokine and/or chemokine gene. The present invention also relates to a product and process for regulating T cell activity by providing a peptide and a superantigen gene, in the presence or absence of a cytokine and/or chemokine gene. In particular, the present invention relates to a product and process for controlling tumor development, immune responses to infectious diseases and diseases caused by immunological disorders.

BACKGROUND OF THE INVENTION

Two major causes of disease include infectious agents and malfunctions of normal biological functions of an animal. Examples of infectious agents include viruses, bacteria, parasites, yeast and other fungi. Examples of abnormal biological function include uncontrolled cell growth, abnormal immune responses and abnormal inflammatory responses. Traditional reagents used attempt to protect an animal from disease include reagents that destroy infectious agents or cells involved in deregulated biological functions. Such reagents, however, can result in unwanted side effects. For example, anti-viral drugs that disrupt the replication of viral DNA also often disrupt DNA replication in normal cells in the treated patient. Other treatments with chemotherapeutic reagents to

destroy cancer cells typically leads to side effects, such as bleeding, vomiting, diarrhea, ulcers, hair loss and increased susceptibility to secondary cancers and infections.

5   An alternative method of disease treatment includes modulating the immune system of a patient to assist the patient's natural defense mechanisms. Traditional reagents and methods used to attempt to regulate an immune response in a patient also result in unwanted side effects and have

10   limited effectiveness. For example, immunosuppressive reagents (e.g., cyclosporin A, azathioprine, and prednisone) used to treat patients with autoimmune disease also suppress the patient's entire immune response, thereby increasing the risk of infection. In addition,

15   immunopharmacological reagents used to treat cancer (e.g., interleukins) are short-lived in the circulation of a patient and are ineffective except in large doses. Due to the medical importance of immune regulation and the inadequacies of existing immunopharmacological reagents,

20   reagents and methods to regulate specific parts of the immune system have been the subject of study for many years.

       Stimulation or suppression of the immune response in a patient can be an effective treatment for a wide variety

25   of medical disorders. T lymphocytes (T cells) are one of a variety of distinct cell types involved in an immune response. The activity of T cells is regulated by antigen, presented to a T cell in the context of a major

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histocompatibility complex (MHC) molecule. The T cell
receptor (TCR) then binds to the MHC:antigen complex. Once
antigen is complexed to MHC, the MHC:antigen complex is
bound by a specific TCR on a T cell, thereby altering the
5   activity of that T cell.

The use of certain staphylococcal enterotoxin proteins
that are capable of complexing with MHC molecules to
influence T cell function has been suggested by various
investigators, including, for example, White et al., *Cell*
10   56:27-35, 1989; Rellahan et al. *J. Expt. Med.* 172:1091-
1100, 1990; Micusan et al., *Immunology* 5:3-11, 1993;
Hermann et al., *Immunology* 5:33-39, 1993; Bhardwaj et al.,
J. Expt. Med. 178:633-642, 1993; and Kalland et al., *Med.
Oncol. & Tumor Pharmacother.*, 10:37-47, 1993.   In
15   particular, various investigators have suggested that
Staphylococcal enterotoxin proteins are useful for treating
tumors, including Newell et al., *Proc. Natl. Acad. Sci. USA*
88:1074-1078, 1991; Kalland et al., PCT Application No. WO
91/04053, published April 4, 1991; Dohlstein et al., *Proc.
20   Natl. Acad. Sci. USA* 88:9287-9291, 1991; Hedlund et al.,
Cancer Immunol. Immunother. 36:89-93, 1993; Lando et al.,
Cancer Immunol. Immunother. 36:223-228, 1993; Lukacs et
al., *J. Exp. Med.* 178:343-348, 1993; Ochi et al., *J.
Immunol.* 151:3180-3186, 1993; and Terman et al., PCT
25   Application No. WO 93/24136, published December 9, 1993.
These investigators, however, have only disclosed the use
of bacterial enterotoxin proteins themselves. The use of

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bacterial enterotoxin protein has the major disadvantage of being toxic to the recipient of the protein.

Thus, there is a need for a product and process that allows for the treatment of disease using bacterial
5   enterotoxins in a non-toxic manner.

SUMMARY

Traditional pharmaceutical reagents used to treat cancer, infectious diseases and diseases caused by
10   immunological disorders often have harmful side effects. In addition, such reagents can be unpredictable (e.g., treatment of cancer, vaccination against infectious agents). For example, chemotherapy and radiotherapy often cause extensive normal tissue damage during the process of
15   treating cancerous tissue. In addition, vaccine treatments for the prevention or cure of infectious diseases are often ineffective because adjuvants useful in vaccine therapy are toxic to an animal.

The present invention is particularly advantageous in
20   that it provides an effective therapeutic composition that enables the safe treatment of an animal with a reagent that is a potentially toxic an immunogenic protein. Upon delivery, expression of acid molecules contained in the therapeutic composition result in localized production of
25   an effective but non-toxic amount of encoded proteins that may be toxic at concentrations that would be required if the encoded proteins were administered directly. The therapeutic compositions of the present invention can

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provide long term expression of the encoded proteins at a site in an animal. Such long term expression allows for the maintenance of an effective, but non-toxic, dose of the encoded protein to treat a disease and limits the frequency
5   of administration of the therapeutic composition needed to treat an animal. In addition, because of the lack of toxicity, therapeutic compositions of the present invention can be used in repeated treatments.

10   BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 illustrates the expression of superantigen-encoding DNA plasmids in mammalian cells.

Fig. 2 illustrates the proliferative response of canine PBMC's to canine melanoma cells transfected with a
15   superantigen-encoding DNA plasmids.

Figs. 3A and 3B illustrate the release of superantigen protein by CHO cells transfected with superantigen-encoding DNA plasmids.

Fig. 4 illustrates the proliferative response of the
20   Vβ3+ T cell clone AD10 to melanoma cells transfected with superantigen-encoding DNA plasmid.

Fig. 5 illustrates the release of canine GM-CSF by CHO cells transfected with GM-CSF-encoding DNA plasmid.

Figs. 6A and 6B illustrate the vaccination of mice
25   with autologous tumor cells transfected with superantigen-encoding DNA plasmid.

Fig. 7 illustrates the effect of tumor target transfection on cytotoxic T cell lysis.

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Fig. 8 illustrates the response of Vβ3+ T cells to intramuscular injection of a superantigen-encoding DNA plasmid.

Fig. 9 illustrates the antibody response resulting
5   from the co-administration of DNA encoding an adjuvant and DNA encoding ovalbumin.

Fig. 10 illustrates that the co-administration of DNA encoding an adjuvant and DNA encoding ovalbumin increase interferon-gamma release from T cells restimulated *in vitro*
10   by the ovalbumin protein.

Fig. 11 illustrates that the co-administration of DNA encoding an adjuvant and DNA encoding ovalbumin increase T cell proliferative responses to ovalbumin.

Fig. 12 illustrates that the co-administration of DNA
15   encoding an adjuvant and DNA encoding ovalbumin increases CTL responses to ovalbumin.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a novel product and
20   process for controlling effector cell activity. The present invention also relates to a novel adjuvant useful for enhancing an immune response. It is now known for the first time that a composition containing nucleic acid molecules encoding a superantigen, rather than superantigen
25   proteins, is an effective therapeutic reagent for treating disease and is an effective adjuvant for enhancing an immune response. As used herein, a disease refers to any biological abnormality that is not beneficial to a subject.

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The present inventors have also discovered that administration of a combination of nucleic acid molecules encoding: (1) a superantigen; (2) a superantigen and a cytokine; or (3) a superantigen and a chemokine, can act

5   synergistically to effectively treat cancer and infectious disease. The present invention includes therapeutic compositions comprising: (a) an isolated nucleic acid molecule encoding a superantigen; or (b) an isolated nucleic acid molecule encoding a superantigen in

10   combination with an isolated nucleic acid molecule encoding a cytokine and/or an isolated nucleic acid molecule encoding a chemokine. Administration of a therapeutic composition of the present invention to an animal results in the production of superantigen, cytokine or chemokine

15   proteins, referred to herein as "encoded proteins." Each of the components of a therapeutic composition of the present invention is described in detail below, followed by a description of the methods by which the therapeutic composition is used and delivered.

20   One embodiment of the present invention includes a method for increasing effector cell immunity in an animal, the method comprising administering to an animal an effective amount of a therapeutic composition comprising: (a) an isolated nucleic acid molecule encoding a

25   superantigen; or (b) an isolated nucleic acid molecule encoding a superantigen in combination with an isolated nucleic acid molecule encoding a cytokine and/or an isolated nucleic acid molecule encoding chemokine.

According to the present embodiment, the nucleic acid molecules are operatively linked to one or more transcription control sequences and the therapeutic composition is targeted to a site in the animal that contains an abnormal cell. According to the present invention, an effector cell, includes a helper T cell, a cytotoxic T cell, a macrophage, a monocyte and/or a natural killer cell. For example, the method of the present invention can be performed to increase the number of effector cells in an animal that are capable of killing or releasing cytokines or chemokines when presented with antigens derived from an abnormal cell or a pathogen. An effective amount of a therapeutic composition of the present invention comprises an amount capable of treating a disease as described herein. Alternatively, a method of the present invention can be performed to decrease the number of T cells found in a T cell subset that is preferentially stimulated and expanded by an autoantigen.

As used herein, effector cell immunity refers to increasing the number and/or the activity of effector cells in the area of the abnormal cell. In particular, T cell activity refers to increasing the number and/or the activity of T cells in the area of the abnormal cell. Also, as used herein, an abnormal cell refers to a cell displaying abnormal biological function, such as abnormal growth, development or death. Abnormal cells of the present invention, preferably includes cancer cells, cells infected with an infectious agent (i.e., a pathogen) and non-

cancerous cells having abnormal proliferative growth (e.g.,
sarcoidosis, granulomatous disease or papillomas) and with
cancer cells and infected cells. Another embodiment of the
present invention is a method to treat an animal with

5   cancer, the method comprising administering to an animal an
effective amount of a therapeutic composition comprising:
(a) a nucleic acid molecule encoding a superantigen; or (b)
a nucleic acid molecule encoding a superantigen in
combination with an isolated nucleic acid molecule encoding

10   a cytokine and/or a nucleic acid molecule encoding a
chemokine.   According to the present embodiment, the
nucleic acid molecules are operatively linked to one or
more transcription control sequences and the therapeutic
composition is targeted to the site of a cancer.

15   One embodiment of a therapeutic composition of the
present invention comprises an isolated nucleic acid
molecule encoding a superantigen (also referred to herein
as a "superantigen-encoding" nucleic acid molecule).
Another embodiment of a therapeutic composition of the

20   present invention comprises an isolated nucleic acid
molecule encoding a superantigen, combined with an isolated
nucleic acid molecule encoding a cytokine (also referred to
herein as a "cytokine-encoding" nucleic acid molecule)
and/or a nucleic acid molecule encoding a chemokine (also

25   referred to as a "chemokine-encoding" nucleic acid
molecule).   According to these embodiments, the nucleic
acid molecules are operatively linked to one or more
transcription control sequences.   It is to be noted that

the term "a" or "an" entity refers to one or more of that entity; for example, a compound refers to one or more compounds. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein.

5   According to the present invention, an isolated, or biologically pure, nucleic acid molecule, is a nucleic acid molecule that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the nucleic acid molecule has

10   been purified. An isolated nucleic acid molecule can include DNA, RNA, or derivatives of either DNA or RNA. An isolated superantigen or cytokine nucleic acid molecule can be obtained from its natural source, either as an entire (i.e., complete) gene or a portion thereof capable of

15   encoding a superantigen protein capable of binding to an MHC molecule or a cytokine protein capable of binding to a complementary cytokine receptor. A nucleic acid molecule can also be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification,

20   cloning) or chemical synthesis. Nucleic acid molecules include natural nucleic acid molecules and homologues thereof, including, but not limited to, natural allelic variants and modified nucleic acid molecules in which nucleotides have been inserted, deleted, substituted,

25   and/or inverted in such a manner that such modifications do not substantially interfere with the nucleic acid molecule's ability to encode a functional superantigen or a functional cytokine of the present invention.

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A nucleic acid molecule homologue can be produced using a number of methods known to those skilled in the art (see, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989).

5   For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis techniques and recombinant DNA techniques, such as site-directed mutagenesis, chemical treatment of a nucleic acid molecule to induce mutations,

10   restriction enzyme cleavage of a nucleic acid fragment, ligation of nucleic acid fragments, polymerase chain reaction (PCR) amplification and/or mutagenesis of selected regions of a nucleic acid sequence, synthesis of oligonucleotide mixtures and ligation of mixture groups to

15   "build" a mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologues can be selected from a mixture of modified nucleic acids by screening for the function of the protein encoded by the nucleic acid (e.g., superantigen, cytokine or chemokine

20   activity, as appropriate). Techniques to screen for superantigen, cytokine or chemokine activity are known to those of skill in the art.

Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase

25   "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being

capable of encoding a superantigen, a cytokine or a chemokine protein. In addition, the phrase "recombinant molecule" primarily refers to a nucleic acid molecule operatively linked to a transcription control sequence, but

5   can be used interchangeably with the phrase "nucleic acid molecule" which is administered to an animal. As heretofore disclosed, superantigen or cytokine proteins of the present invention include, but are not limited to, proteins having full-length superantigen, cytokine or chemokine coding

10   regions, proteins having partial superantigen regions capable of binding to an MHC molecule, cytokine coding regions capable of binding to a complementary cytokine receptor, chemokine coding regions capable of binding to a complementary chemokine receptor, fusion proteins and

15   chimeric proteins comprising combinations of different superantigens, cytokines and/or chemokines.

One embodiment of the present invention is an isolated superantigen-encoding nucleic acid molecule that encodes at least a portion of a full-length superantigen, or a

20   homologue of a superantigen. As used herein, "at least a portion of a superantigen" refers to a portion of a superantigen protein capable of binding to an MHC molecule in such a manner that a TCR can bind to the resulting superantigen:MHC complex. Preferably, a superantigen

25   nucleic acid molecule of the present invention encodes an entire coding region of a superantigen, and more preferably the coding region absent a leader sequence. Production of a truncated superantigen protein lacking a bacterial leader

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sequence is preferred to enhance secretion of the superantigen from a cell. As used herein, a homologue of a superantigen is a protein having an amino acid sequence that is sufficiently similar to a natural superantigen

5   amino acid sequence that a nucleic acid sequence encoding the homologue encodes a protein capable of binding to an MHC molecule.

In accordance with the present invention, a superantigen comprises a family of T cell regulatory

10 proteins that are capable of binding both to an MHC molecule. A superantigen binds to the extracellular portion of an MHC molecule to form and MHC:superantigen complex. The activity of a T cell can be modified when a TCR binds to an MHC:superantigen complex. Under certain

15 circumstances, an MHC:superantigen complex can have a mitogenic role (i.e., the ability to stimulate the proliferation of T cells) or a suppressive role (i.e., deletion of T cell subsets). The ability of an MHC:superantigen complex to have a stimulatory or

20 suppressive role can depend upon factors, such as the concentration and environment (i.e., tissue location and/or the presence of cytokines).

The mitogenic role of a superantigen is distinct from other known mitogens (e.g., lectins derived from plants) in

25 that superantigens are capable of stimulating the proliferation of particular subsets of T cells having TCR's that specifically bind to the superantigen. For example, a superantigen, when added to a mixed lymphocyte

population, is able to stimulate the proliferation of a
select population of T cells from the mixed population of
cells. Examples of T cell subsets stimulated by
superantigens complexed with MHC molecules include T cells

5   expressing a TCR comprising mouse $V_\beta 1$, $V_\beta 3$, $V_\beta 7$, $V_\beta 8.1$,
$V_\beta 8.2$, $V_\beta 8.3$, $V_\beta 10$, $V_\beta 11$, $V_\beta 17$, $V_\beta 15$ or $V_\beta 16$ chains, and T
cells expressing a TCR comprising human $V_\beta 1.1$, $V_\beta 2$, $V_\beta 3$, $V_\beta 5$,
$V_\beta 6$, $V_\beta 7.3$, $V_\beta 7.4$, $V_\beta 9.1$, $V_\beta 12$, $V_\beta 14$, $V_\beta 15$, $V_\beta 17$ or $V_\beta 20$
chains.

10   A superantigen-encoding nucleic acid molecule of the
present invention preferably encodes superantigens that
includes, but is not limited to, staphylococcal
enterotoxins, retroviral antigens, streptococcal antigens,
mycoplasma antigens, mycobacterium antigens, viral antigens

15 (e.g., a superantigen from mouse mammary tumor virus,
rabies virus or herpes virus) and endoparasitic antigens
(e.g., protozoan or helminth antigens), more preferably
staphylococcal enterotoxins, and even more preferably
Staphylococcal enterotoxin A (SEA), *Staphylococcal*

20 *enterotoxin* B (SEB), *Staphylococcal enterotoxin* C_1 (SEC_1),
Staphylococcal enterotoxin C_2 (SEC_2), *Staphylococcal*
enterotoxin C_3 (SEC_3), *Staphylococcal enterotoxin* D (SED),
Staphylococcal enterotoxin E (SEE) and Toxic Shock Syndrome
Toxin (TSST).

25   A preferred nucleic acid molecule encoding a
Staphylococcal enterotoxin of the present invention
comprises a nucleic acid sequence represented by SEQ ID
NO:1 (representing a full-length SEB gene), SEQ ID NO:3

(representing a full-length SEA gene) or SEQ ID NO:6
(representing a full-length TSST gene). A preferred
Staphylococcal enterotoxin protein of the present invention
comprises an amino acid sequence represented by SEQ ID NO:2

5   (representing a full-length SEB protein), SEQ ID NO:4
(representing a full-length SEA protein) or SEQ ID NO:7
(representing a full-length TSST protein).

In a preferred embodiment, a nucleic acid molecule of
the present invention encoding a superantigen comprises a

10   nucleic acid sequence spanning base pair 46 to at least
base pair 768 of SEQ ID NO:1, a nucleic acid sequence
spanning base pair 46 to about base pair 751 of SEQ ID NO:3
or SEQ ID NO:6.

Another embodiment of the present invention includes

15   a cytokine-encoding nucleic acid molecule that encodes a
full-length cytokine or a homologue of the cytokine
protein. As used herein, a homologue of a cytokine is a
protein having an amino acid sequence that is sufficiently
similar to a natural cytokine amino acid sequence so as to

20   have cytokine activity. In accordance with the present
invention, a cytokine includes a protein that is capable of
affecting the biological function of another cell. A
biological function affected by a cytokine can include, but
is not limited to, cell growth, cell differentiation or

25   cell death. Preferably, a cytokine of the present
invention is capable of binding to a specific receptor on
the surface of a cell, thereby affecting the biological
function of a cell.

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A cytokine-encoding nucleic acid molecule of the present invention encodes a cytokine that is capable of affecting the biological function of a cell, including, but not limited to, a lymphocyte, a muscle cell, a

5 hematopoietic precursor cell, a mast cell, a natural killer cell, a macrophage, a monocyte, an epithelial cell, an endothelial cell, a dendritic cell, a mesenchymal cell, a Langerhans cell, cells found in granulomas and tumor cells of any cellular origin, and more preferably a mesenchymal

10 cell, an epithelial cell, an endothelial cell, a muscle cell, a macrophage, a monocyte, a T cell and a dendritic cell.

A preferred cytokine nucleic acid molecule of the present invention encodes a hematopoietic growth factor, an

15 interleukin, an interferon, an immunoglobulin superfamily molecule, a tumor necrosis factor family molecule and/or a chemokine (i.e., a protein that regulates the migration and activation of cells, particularly phagocytic cells). A more preferred cytokine nucleic acid molecule of the

20 present invention encodes a granulocyte macrophage colony stimulating factor (GM-CSF), tumor necrosis factor α (TNF-α), macrophage colony stimulating factor (M-CSF), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-12 (IL-12),

25 interleukin-15 (IL-15) and/or IGIF. An even more preferred cytokine nucleic acid molecule of the present invention encodes GM-CSF, IL-2, IL-12, IGIF and/or TNF-α, with GM-CSF being even more preferred.

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As will be apparent to one of skill in the art, the present invention is intended to apply to cytokines derived from all types of animals. A preferred animal from which to derive cytokines includes a mouse, a human, a cat and a

5   dog. A more preferred animal from which to derive cytokines includes a cat, a dog and a human. An even more preferred animal from which to derive cytokines is a human.

According to the present invention, a cytokine-encoding nucleic acid molecule of the present invention is

10   derived from the same species of animal as the animal to be treated. For example, a cytokine-encoding nucleic acid molecule derived from a canine (i.e., dog) nucleic acid molecule is used to treat a disease in a canine. Thus, a preferred cytokine-encoding nucleic acid molecule of the

15   present invention comprises a nucleic acid molecule encoding human GM-CSF, as described in the art. A human GM-CSF-encoding nucleic acid molecule of the present invention can be produced using methods standard PCR amplification methods with primers designed from the human

20   GM-CSF nucleic acid sequence disclosed in Nash (*Blood* 78:930, 1991). Such PCR products can be cloned into a PCR$_3$ expression vector using the methods generally described in Example 1.

Another embodiment of the present invention includes

25   a chemokine-encoding nucleic acid molecule that encodes a full-length chemokine or a homologue of the chemokine protein. As used herein, a homologue of a chemokine is a protein having an amino acid sequence that is sufficiently

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similar to a natural chemokine amino acid sequence so as to have chemokine activity. In accordance with the present invention, a chemokine includes a protein that is capable of attracting cells involved in an immune response

5   (immunologic cells), including phagocytic cells. For example, immunologic cells are recruited from the blood to a site at which the chemokine is located (e.g., a site of infection). Preferably, a chemokine of the present invention is capable of binding to a specific receptor on

10   the surface of a cell, thereby attracting the cell to a specific location.

A chemokine-encoding nucleic acid molecule of the present invention encodes a chemokine that is capable of attracting a cell to a site, including, but not limited to,

15   a dendritic cell, a neutrophil, a macrophage, a T lymphocyte and Langerhans cells, and more preferably a dendritic cell, a macrophage and a T lymphocyte.

A preferred chemokine-encoding nucleic acid molecule of the present invention encodes an α-chemokine or a β-

20   chemokine. A more preferred chemokine-encoding nucleic acid molecule of the present invention encodes a C5a, interleukin-8 (IL-8), monocyte chemotactic protein 1α (MIP1α), monocyte chemotactic protein 1β (MIP1β), monocyte chemoattractant protein 1 (MCP-1), monocyte chemoattractant

25   protein 3 (MCP-3), platelet activating factor (PAFR), N-Formyl-methionyl-leucyl-[^{3H}]phenylalanine (FMLPR), leukotriene B$_4$ (LTB$_4$R), gastrin releasing peptide (GRP), RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2,

NAP-2 and/or MGSA/gro. An even more preferred chemokine-
encoding nucleic acid molecule of the present invention
encodes IL-8, MIP1α, MIP1β, MCP-1, MCP-3, RANTES and/or
NAP-2, with IL-8, Rantes, MIP1α and/or MIP1β being even
5   more preferred.

        As will be apparent to one of skill in the art, the
present invention is intended to apply to chemokines
derived from all types of animals. Preferred animals from
which to derive chemokines includes mice, humans, dogs,
10   cats, cattle and horses. More preferred animals from which
to derive chemokines includes dogs, cats, humans and
cattle. Even more preferred animals from which to derive
chemokines are humans.

        According to the present invention, a chemokine-
15   encoding nucleic acid molecule of the present invention is
derived from the same species of animal as the animal to be
treated. For example, a chemokine-encoding nucleic acid
molecule derived from a canine (i.e., dog) nucleic acid
molecule is used to treat a disease in a canine. Thus, a
20   preferred chemokine-encoding nucleic acid molecule of the
present invention comprises a nucleic acid molecule
encoding a dog, cat, human, bovine and/or equine chemokine.
Preferred nucleic acid molecules of the present invention
encode IL-8, Rantes, MIP1α and/or MIP1β, as described in
25   the art. For example, a human MIP1α-encoding nucleic acid
molecule of the present invention can be produced using
standard PCR amplification methods with primers designed
from the human MIP1α-encoding nucleic acid sequence

disclosed in the art. Such PCR products can be cloned into a PCR$_3$ expression vector using the methods generally described in Example 1.

The present invention includes a nucleic acid molecule

5   of the present invention operatively linked to one or more transcription control sequences to form a recombinant molecule. The phrase "operatively linked" refers to linking a nucleic acid molecule to a transcription control sequence in a manner such that the molecule is able to be

10 expressed when transfected (i.e., transformed, transduced or transfected) into a host cell. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which

15 control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of

20 such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in animal, bacteria, helminth, insect cells, and preferably in animal cells. More preferred transcription control sequences include, but

25 are not limited to, simian virus 40 (SV-40), β-actin, retroviral long terminal repeat (LTR), Rous sarcoma virus (RSV), cytomegalovirus (CMV), *tac, lac, trp, trc,* oxy-pro, omp/lpp, rrnB, bacteriophage lambda (λ) (such as λp_L and λp_R

and fusions that include such promoters), bacteriophage T7,
T7*lac*, bacteriophage T3, bacteriophage SP6, bacteriophage
SP01, metallothionein, alpha mating factor, *Pichia* alcohol
oxidase, alphavirus subgenomic promoters (such as Sindbis

5   virus subgenomic promoters), baculovirus, *Heliothis zea*
insect virus, vaccinia virus and other poxviruses,
herpesvirus, and adenovirus transcription control
sequences, as well as other sequences capable of
controlling gene expression in eukaryotic cells.

10 Additional suitable transcription control sequences include
tissue-specific promoters and enhancers (e.g., tumor cell-
specific enhancers and promoters), and inducible promoters
(e.g., tetracycline). Transcription control sequences of
the present invention can also include naturally occurring

15 transcription control sequences naturally associated with
a gene encoding a superantigen, a cytokine or a chemokine
of the present invention.

        Recombinant molecules of the present invention, which
can be either DNA or RNA, can also contain additional

20 regulatory sequences, such as translation regulatory
sequences, origins of replication, and other regulatory
sequences that are compatible with the recombinant cell.
In one embodiment, a recombinant molecule of the present
invention also contains secretory signals (i.e., signal

25 segment nucleic acid sequences) to enable an expressed
superantigen, cytokine or a chemokine protein to be
secreted from the cell that produces the protein. Suitable
signal segments include: (1) a bacterial signal segment, in

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particular a superantigen signal segment; (2) a cytokine
signal segment; (3) a chemokine signal segment; (4) or any
heterologous signal segment capable of directing the
secretion of a superantigen, cytokine and/or chemokine
5   protein of the present invention.   Preferred signal
segments include, but are not limited to, signal segments
associated with SEB, SEA, TSST, GM-CSF, M-CSF, TNFα, IL-1,
IL-2, IL-4, IL-6, IL-12, IL-15, C5a, IGIF, IL-8, MIP1α,
MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB$_4$R, GRP, RANTES,
10   eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2
and/or MGSA/gro protein.

Preferred recombinant molecules of the present
invention include a recombinant molecule containing a
nucleic acid molecule encoding a superantigen, a
15   recombinant molecule containing a nucleic acid molecule
encoding a cytokine, a recombinant molecule containing a
nucleic acid molecule encoding a chemokine, a recombinant
molecule containing a nucleic acid molecule encoding a
superantigen and a nucleic acid molecule encoding a
20   cytokine to form a chimeric recombinant molecule, or a
recombinant molecule containing a nucleic acid molecule
encoding a superantigen and a nucleic acid molecule
encoding a chemokine to form a chimeric recombinant
molecule.   The nucleic acid molecules contained in such
25   recombinant chimeric molecules are operatively linked to
one or more transcription control sequences, in which each
nucleic acid molecule contained in a chimeric recombinant
molecule can be expressed using the same or different

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regulatory control sequences. Preferred recombinant molecules of the present invention comprise a nucleic acid sequence represented by SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or combinations thereof. Particularly preferred

5   recombinant molecules include PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST and PCR_3-GM_3, the production of which is disclosed herein. Other preferred nucleic acid sequences include Rantes nucleic acid sequence (SEQ ID. NO:13), MIP1α nucleic acid sequence (see Davatelis et al., *J. Exp. Med.*

10   167:1939-1944, 1988) and MIP1β nucleic acid sequence (see Sherry et al., *J. Exp. Med.* 168:2251-2259, 1988).

According to the present invention, a recombinant molecule can be dicistronic. A cistron refers to a unit of DNA that is capable of encoding an amino acid sequence

15   having a naturally-occurring biological function. A dicistronic plasmid refers to a plasmid containing 2 cistrons. Preferably, a dicistronic recombinant molecule of the present invention comprises an internal ribosome entry site (IRES) element to which eukaryotic ribosomes can

20   bind (see, for example, Jang et al., *J. Virol.* 62:2636-2643, 1988; Pelletier et al. *Nature* 334:320-325, 1988; Jackson, *Nature* 353:14-15, 1991; Macejek et al., *Nature* 353:90-94, 1991; Oh et al., *Genes & Develop.* 6:1643-1653, 1992; Molla et al., *Nature* 356:255-257, 1992; and Kozak,

25   *Crit. Rev. Biochem. Molec. Biol.* 27(4,5):385-402, 1992).

In one embodiment, a dicistronic recombinant molecule of the present invention comprises a eukaryotic promoter, operatively linked to a superantigen-encoding nucleic acid

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molecule of the present invention and a cytokine-encoding nucleic acid molecule of the present invention separated by an IRES nucleic acid sequence, or a superantigen-encoding nucleic acid molecule of the present invention and

5   chemokine-encoding nucleic acid molecule of the present invention separated by an IRES nucleic acid sequence.

In another embodiment, a dicistronic recombinant molecule of the present invention comprises a eukaryotic promoter, operatively linked to a first superantigen-

10   encoding nucleic acid molecule of the present invention and a second superantigen-encoding nucleic acid molecule of the present invention separated by an IRES nucleic acid sequence. Preferably, a first superantigen-encoding nucleic acid molecule encodes a different superantigen than

15   a second superantigen-encoding nucleic acid molecule.

One or more recombinant molecules of the present invention can be used to produce an encoded product (i.e., a superantigen protein, a cytokine and a chemokine protein) of the present invention. In one embodiment, an encoded

20   product of the present invention is produced by expressing a nucleic acid molecule of the present invention in a cell under conditions effective to produce the protein. A preferred method to produce an encoded protein is by transforming (i.e., introducing a recombinant molecule into

25   a cell in such a manner that the recombinant molecule is expressed by the cell) a host cell with one or more recombinant molecules of the present invention to form a recombinant cell. Suitable host cells to transform include

any cell into which a recombinant molecule can be introduced. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule. Host cells of the present invention

5   can be any cell capable of producing a superantigen, a cytokine and/or a chemokine of the present invention, including bacterial, fungal, animal parasite, insect and animal cells. A preferred host cell includes a mammalian and a bird cell. A more preferred host cell includes

10   mammalian lymphocytes, muscle cells, hematopoietic precursor cells, mast cells, natural killer cells, macrophages, monocytes, epithelial cells, endothelial cells, dendritic cells, mesenchymal cells, Langerhans cells, cells found in granulomas and tumor cells of any

15   cellular origin. An even more preferred host cell of the present invention includes mammalian mesenchymal cells, epithelial cells, endothelial cells, macrophages, monocytes, muscle cells, T cells and dendritic cells.

        According to the present invention, a recombinant

20   molecule can be introduced into a host cell *in vivo* (i.e., in an animal) or *in vitro* (i.e., outside of an animal, such as in tissue culture). Introduction of a nucleic acid molecule into a host cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the

25   cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. Preferred methods to introduce a recombinant molecule into host cells

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in vivo include lipofection and adsorption (discussed in detail below).

A recombinant cell of the present invention comprises a cell into which a nucleic acid molecule that encodes a
5   superantigen, a cytokine and/or a chemokine has been introduced. In one embodiment, a recombinant cell of the present invention is transformed with a nucleic acid molecule that includes at least a portion of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST, or combinations
10 thereof. Particularly preferred recombinant cells include cells transformed with PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S or PCR_3-TSST, with PCR_3-SEB.S, PCR_3-SEA.S or PCR_3-TSST being even more preferred.

In another embodiment, a recombinant cell of the
15 present invention is transformed with a nucleic acid molecule that includes at least a portion of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST or combinations thereof, and PCR_3-GM_3. Particularly preferred stimulatory recombinant cells include cells transformed with PCR_3-SEA
20 and PCR_3-GM_3, PCR_3-SEA.S and PCR_3-GM_3, PCR_3-SEB and PCR_3-GM_3, PCR_3-SEB.S and PCR_3-GM_3, or PCR_3-TSST and PCR_3-GM_3. Even more preferred stimulatory recombinant cells include cells transformed with PCR_3-SEB.S and PCR_3-GM_3, or PCR_3-SEA.S and PCR_3-GM_3, and PCR_3-TSST and PCR_3-GM_3.

25 Recombinant DNA technologies can be used to improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency

with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing

5   the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector

10 stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences),

15 modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, and deletion of sequences that destabilize transcripts. The activity of an expressed recombinant protein of the present invention may be improved by

20 fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

Further embodiments of compositions of the present invention can also include a compound capable of inhibiting the downregulation of T cell activity. In particular, such

25 a compound can include an inhibitor of CTLA-4. An inhibitor of CTLA-4 includes any compound capable of inhibiting the activity of CTLA-4 and/or inhibiting the binding of CTLA-4 to its natural ligand (e.g., B7).

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Preferably, an inhibitor of CTLA-4 includes, but is not limited to a ligand of CTLA-4 or an analog (i.e., antagonist) of CTLA-4. Preferred ligands of CTLA-4 include: an antibody that specifically binds to CTLA-4 in

5   such a manner that CTLA-4 activity is inhibited; at least a portion of a B7 molecule, in particular a B7 fusion protein; or a synthetic oligonucleotide that binds CTLA-4 protein. A preferred analog of CTLA-4 includes a molecule capable of binding to B7 in such a manner that B7 signal

10   transduction is not activated and CTLA-4 binding to the B7 molecule is inhibited. It is within the scope of the invention that a CTLA-4 inhibitor can comprise a nucleic acid molecule, a protein or a synthetic chemical molecule when combined in a composition of the present invention.

15   In another embodiment of the present invention, a therapeutic composition further comprises a pharmaceutically acceptable carrier. As used herein, a "carrier" refers to any substance suitable as a vehicle for delivering a nucleic acid molecule of the present invention

20   to a suitable *in vivo* or *in vitro* site. As such, carriers can act as a pharmaceutically acceptable excipient of a therapeutic composition containing a nucleic acid molecule of the present invention. Preferred carriers are capable of maintaining a nucleic acid molecule of the present

25   invention in a form that, upon arrival of the nucleic acid molecule to a cell, the nucleic acid molecule is capable of entering the cell and being expressed by the cell. Carriers of the present invention include: (1) excipients

or formularies that transport, but do not specifically target a nucleic acid molecule to a cell (referred to herein as non-targeting carriers); and (2) excipients or formularies that deliver a nucleic acid molecule to a

5   specific site in an animal or a specific cell (i.e., targeting carriers). Examples of non-targeting carriers include, but are not limited to water, phosphate buffered saline, Ringer's solution, dextrose solution, serum-containing solutions, Hank's solution, other aqueous

10 physiologically balanced solutions, oils, esters and glycols. Aqueous carriers can contain suitable auxiliary substances required to approximate the physiological conditions of the recipient, for example, by enhancing chemical stability and isotonicity.

15   Suitable auxiliary substances include, for example, sodium acetate, sodium chloride, sodium lactate, potassium chloride, calcium chloride, and other substances used to produce phosphate buffer, Tris buffer, and bicarbonate buffer. Auxiliary substances can also include

20 preservatives, such as thimerosal, m- and o-cresol, formalin and benzol alcohol. Preferred auxiliary substances for aerosol delivery include surfactant substances non-toxic to an animal, for example, esters or partial esters of fatty acids containing from about six to

25 about twenty-two carbon atoms. Examples of esters include, caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric, and oleic acids. Other carriers can include metal particles (e.g., gold particles) for use

with, for example, a biolistic gun through the skin. Therapeutic compositions of the present invention can be sterilized by conventional methods and/or lyophilized.

Targeting carriers are herein referred to as "delivery
5   vehicles." Delivery vehicles of the present invention are capable of delivering a therapeutic composition of the present invention to a target site in an animal. A "target site" refers to a site in an animal to which one desires to deliver a therapeutic composition. For example, a target
10 site can be a malignant tumor cell, a non-malignant tumor cell, a lymph node or a lesion caused by an infectious agent, or an area around such cell, tumor or lesion, which is targeted by direct injection or delivery using liposomes or other delivery vehicles. Examples of delivery vehicles
15 include, but are not limited to, artificial and natural lipid-containing delivery vehicles. Natural lipid-containing delivery vehicles include cells and cellular membranes. Artificial lipid-containing delivery vehicles include liposomes and micelles. A delivery vehicle of the
20 present invention can be modified to target to a particular site in an animal, thereby targeting and making use of a nucleic acid molecule of the present invention at that site. Suitable modifications include manipulating the chemical formula of the lipid portion of the delivery
25 vehicle and/or introducing into the vehicle a compound capable of specifically targeting a delivery vehicle to a preferred site, for example, a preferred cell type. Specifically targeting refers to causing a delivery vehicle

to bind to a particular cell by the interaction of the compound in the vehicle to a molecule on the surface of the cell. Suitable targeting compounds include ligands capable of selectively (i.e., specifically) binding another

5   molecule at a particular site. Examples of such ligands include antibodies, antigens, receptors and receptor ligands. For example, an antibody specific for an antigen found on the surface of a cancer cell can be introduced to the outer surface of a liposome delivery vehicle so as to

10   target the delivery vehicle to the cancer cell. Tumor cell ligands include ligands capable of binding to a molecule on the surface of a tumor cell. Manipulating the chemical formula of the lipid portion of the delivery vehicle can modulate the extracellular or intracellular targeting of

15   the delivery vehicle. For example, a chemical can be added to the lipid formula of a liposome that alters the charge of the lipid bilayer of the liposome so that the liposome fuses with particular cells having particular charge characteristics.

20   A preferred delivery vehicle of the present invention is a liposome. A liposome is capable of remaining stable in an animal for a sufficient amount of time to deliver a nucleic acid molecule of the present invention to a preferred site in the animal. A liposome of the present

25   invention is preferably stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour and even more preferably for at least about 24 hours.

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A liposome of the present invention comprises a lipid composition that is capable of targeting a nucleic acid molecule of the present invention to a particular, or selected, site in an animal. Preferably, the lipid

5   composition of the liposome is capable of targeting to any organ of an animal, more preferably to the lung, liver, spleen, heart brain, lymph nodes and skin of an animal, and even more preferably to the lung of an animal.

A liposome of the present invention comprises a lipid

10   composition that is capable of fusing with the plasma membrane of the targeted cell to deliver a nucleic acid molecule into a cell. Preferably, the transfection efficiency of a liposome of the present invention is at least about 0.5 microgram (μg) of DNA per 16 nanomole

15   (nmol) of liposome delivered to about 10^6 cells, more preferably at least about 1.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells, and even more preferably at least about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells.

20   A preferred liposome of the present invention is between about 100 and about 500 nanometers (nm), more preferably between about 150 and about 450 nm and even more preferably between about 200 and about 400 nm in diameter.

Suitable liposomes for use with the present invention

25   include any liposome. Preferred liposomes of the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes comprise liposomes

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having a polycationic lipid composition and/or liposomes
having a cholesterol backbone conjugated to polyethylene
glycol.   Even more preferred liposomes include liposomes
produced according to the method described in Example 2.

5   In one embodiment, a liposome of the present invention
comprises a compound capable of targeting the liposome to
a tumor cell.   Such a liposome preferably includes a tumor
cell ligand exposed on the outer surface of the liposome.

         Complexing a liposome with a nucleic acid molecule of
10   the present invention can be achieved using methods
standard in the art (see, for example, methods described in
Example 2).   A suitable concentration of a nucleic acid
molecule of the present invention to add to a liposome
includes a concentration effective for delivering a
15   sufficient amount of nucleic acid molecule to a cell such
that the cell can produce sufficient superantigen and/or
cytokine protein to regulate effector cell immunity in a
desired manner.   Preferably, nucleic acid molecules are
combined with liposomes at a ratio of from about 0.1 μg to
20   about 10 μg of nucleic acid molecule of the present
invention per about 8 nmol liposomes, more preferably from
about 0.5 μg to about 5 μg of nucleic acid molecule per
about 8 nmol liposomes, and even more preferably about 1.0
μg of nucleic acid molecule per about 8 nmol liposomes.

25   Another preferred delivery vehicle comprises a
recombinant virus particle vaccine.   A recombinant virus
particle vaccine of the present invention includes a
therapeutic composition of the present invention, in which

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the recombinant molecules contained in the composition are packaged in a viral coat that allows entrance of DNA into a cell so that the DNA is expressed in the cell. A number of recombinant virus particles can be used, including, but

5   not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, arena virus and retroviruses.

Another preferred delivery vehicle comprises a recombinant cell vaccine. Preferred recombinant cell vaccines of the present invention include tumor vaccines,

10   in which allogeneic (i.e., cells derived from a source other than a patient, but that are histiotype compatible with the patient) or autologous (i.e., cells isolated from a patient) tumor cells are transfected with recombinant molecules contained in a therapeutic composition,

15   irradiated and administered to a patient by, for example, intradermal, intravenous or subcutaneous injection. Therapeutic compositions to be administered by tumor cell vaccine, include recombinant molecules of the present invention without carrier. Tumor cell vaccine treatment is

20   useful for the treatment of both tumor and metastatic cancer. Use of a tumor vaccine of the present invention is particular useful for treating metastatic cancer, including preventing metastatic disease, as well as, curing existing metastatic disease. Methods for developing and

25   administering include those standard in the art (see for example, Dranoff et al., *Proc. Natl. Acad. Sci. USA* 90:3539-3543, 1993, which is incorporated herein by reference in its entirety).

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A therapeutic composition of the present invention is useful for the treatment of a variety of diseases, including, but not limited to, cancer, autoimmune disease, infectious diseases, and other diseases that can be

5   alleviated by either stimulating or suppressing T cell activity. As used herein, the term "treatment" refers to protecting an animal from a disease or alleviating a disease in an animal. A therapeutic composition of the present invention is advantageous for the treatment of

10 cancer in that the composition overcomes the mechanisms by which cancer cells avoid immune elimination (i.e., by which cancer cells avoid the immune response effected by the animal in response to the disease). Cancer cells can avoid immune elimination by, for example, being only slightly

15 immunogenic, modulating cell surface antigens and inducing immune suppression. Suitable therapeutic compositions for use in the treatment of cancer comprises a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a

20 cytokine-encoding recombinant molecule and/or a chemokine recombinant molecule of the present invention. Preferred therapeutic compositions for use in the treatment of cancer comprises a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant

25 molecule with a cytokine-encoding recombinant molecule and/or a chemokine recombinant molecule of the present invention combined (separately or together) with a delivery vehicle, preferably a liposome, such as disclosed herein.

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A therapeutic composition of the present invention, upon entering targeted cells, leads to the production of superantigen, cytokine and/or chemokine protein that activate cytotoxic T cells, natural killer cells, T helper cells and macrophages. Such cellular activation overcomes the otherwise relative lack of immune response to cancer cells, leading to the destruction of such cells.

A therapeutic composition of the present invention is useful for the treatment of cancers, both tumors and metastatic forms of cancer. Treatment with the therapeutic composition overcomes the disadvantages of traditional treatments for metastatic cancers. For example, compositions of the present invention can target dispersed metastatic cancer cells that cannot be treated using surgical methods. In addition, administration of such compositions do not result in the harmful side effects caused by chemotherapy and radiation therapy.

A therapeutic composition of the present invention is preferably used to treat cancers, including, but not limited to, melanomas, squamous cell carcinoma, breast cancers, head and neck carcinomas, thyroid carcinomas, soft tissue sarcomas, bone sarcomas, testicular cancers, prostatic cancers, ovarian cancers, bladder cancers, skin cancers, brain cancers, angiosarcomas, hemangiosarcomas, mast cell tumors, primary hepatic cancers, lung cancers, pancreatic cancers, gastrointestinal cancers, renal cell carcinomas, hematopoietic neoplasias, leukemias and lymphomas. Particularly preferred cancers to treat with a

therapeutic composition of the present invention, include melanomas, lung cancers, thyroid carcinomas, breast cancers, renal cell carcinomas, squamous cell carcinomas, brain tumors and skin cancers. A therapeutic composition

5   of the present invention is useful for treating tumors that can form in such cancers, including malignant and benign tumors.

A therapeutic composition of the present invention is also advantageous for the treatment of infectious diseases

10 as a long term, targeted therapy for primary lesions (e.g., granulomas) resulting from the propagation of a pathogen. As used herein, the term "lesion" refers to a lesion formed by infection of an animal with a pathogen. Preferred therapeutic compositions for use in the treatment of an

15 infectious disease comprise a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine recombinant molecule of the present invention. More preferred

20 therapeutic compositions for use in the treatment of infectious disease comprise a superantigen-encoding recombinant molecule; or a combination of superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine recombinant

25 molecule of the present invention combined with a delivery vehicle, preferably a liposome of the present invention. Similar to the mechanism described for the treatment of cancer, treatment of infectious diseases with superantigen,

cytokine and/or chemokine can result in increased T cell, natural killer cell, and macrophage cell activity that overcome the relative lack of immune response to a lesion formed by a pathogen.

5   A therapeutic composition of the present invention is particularly useful for the treatment of infectious diseases caused by pathogens, including, but not limited to, intracellular bacteria (i.e., a bacteria that resides in a host cell), internal parasites, pathogenic fungi and

10   endoparasites. Particularly preferred infectious diseases to treat with a therapeutic composition of the present invention include tuberculosis, leprosy, aspergillosis, coccidioidomycosis, cryptococcoses, leishmaniasis and toxoplasmosis.

15   In order to treat an animal with disease, a therapeutic composition of the present invention is administered to the animal in an effective manner such that the composition is capable of treating that animal from disease. For example, a recombinant molecule, when

20   administered to an animal in an effective manner, is able to stimulate effector cell immunity in a manner that is sufficient to alleviate the disease afflicting the animal. According to the present invention, treatment of a disease refers to alleviating a disease and/or preventing the

25   development of a secondary disease resulting from the occurrence of a primary disease.

An   effective   administration   protocol   (i.e., administering a therapeutic composition in an effective

manner) comprises suitable dose parameters and modes oᵢ administration that result in treatment of a disease. Effective dose parameters and modes of administration can be determined using methods standard in the art for a particular disease. Such methods include, for example, determination of survival rates, side effects (i.e., toxicity) and progression or regression of disease. In particular, the effectiveness of dose parameters and modes of administration of a therapeutic composition of the present invention when treating cancer can be determined by assessing response rates. Such response rates refer to the percentage of treated patients in a population of patients that respond with either partial or complete remission. Remission can be determined by, for example, measuring tumor size or microscopic examination for the presence of cancer cells in a tissue sample.

In accordance with the present invention, a suitable single dose size is a dose that is capable of treating an animal with disease when administered one or more times over a suitable time period. Doses can vary depending upon the disease being treated. In the treatment of cancer, a suitable single dose can be dependent upon whether the cancer being treated is a primary tumor or a metastatic form of cancer. Doses of a therapeutic composition of the present invention suitable for use with direct injection techniques can be used by one of skill in the art to determine appropriate single dose sizes for systemic administration based on the size of an animal. A suitable

single dose of a therapeutic composition to treat a tumor is a sufficient amount of a superantigen-encoding recombinant molecule; or a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant

5   molecule and/or a chemokine recombinant molecule to reduce, and preferably eliminate, the tumor following transfection of the recombinant molecules into cells at or near the tumor site. A preferred single dose of the superantigen-encoding recombinant molecule is an amount that, when

10   transfected into a target cell population, leads to the production of from about 250 femtograms (fg) to about 1 μg, preferably from about 500 fg to about 500 picogram (pg), and more preferably from about 1 pg to about 100 pg of superantigen per transfected cell. A preferred single dose

15   of a cytokine-encoding recombinant molecule is an amount that, when transfected into a target cell population, leads to the production of from about 10 pg to about 1 μg, preferably from about 100 pg to about 750 pg, and more preferably about 500 pg of cytokine per transfectant. A

20   preferred single dose of a chemokine-encoding recombinant molecule is an amount that, when transfected into a target cell population, leads to the production of from about 1 fg to about 1 μg, preferably from about 1 pg to about 10 ng, and more preferably from about 1 pg to about 1 ng chemokine

25   per transfectant.

A suitable single dose of a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding

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recombinant molecule and/or a chemokine-encoding recombinant molecule in a non-targeting carrier to administer to an animal to treat a tumor, is an amount capable of reducing, and preferably eliminating, the tumor

5   following transfection of the recombinant molecules into cells at or near the tumor site. A preferred single dose of a therapeutic composition to treat a tumor is from about 100 μg to about 2 milligrams (mg) of total recombinant molecules, more preferably from about 150 μg to about 1 mg

10   of total recombinant molecules, and even more preferably from about 200 μg to about 800 μg of total recombinant molecules. A preferred single dose of a superantigen-encoding recombinant molecule complexed with liposomes, is from about 100 μg of total DNA per 800 nmol of liposome to

15   about 2 mg of total recombinant molecules per 16 micromole (μmol) of liposome, more preferably from about 150 μg per 1.2 μmol of liposome to about 1 mg of total recombinant molecules per 8 μmol of liposome, and even more preferably from about 200 μg per 2 μmol of liposome to about 400 μg of

20   total recombinant molecules per 3.2 μmol of liposome.

A preferred single dose of a cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule in a non-targeting carrier to administer to an animal to treat a tumor, is from about 100 μg to about 2 mg

25   of total recombinant molecules, more preferably from about 150 μg to about 1 mg of total recombinant molecules, and even more preferably from about 200 μg to about 400 μg of total recombinant molecules. A preferred single dose of a

cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule complexed with liposomes to administer to an animal to treat a tumor, is from about 100 μg of total recombinant molecules per 800 nmol of liposome

5   to about 2 mg of total recombinant molecules per 16 μmol of liposome, more preferably from about 150 μg per 1.2 μmol of liposome to about 1 mg of total recombinant molecules per 8 μmol of liposome, and even more preferably from about 200 μg per 2 μmol of liposome to about 400 μg of total

10 recombinant molecules per 6.4 μmol of liposome.

A preferred single dose of a superantigen-encoding recombinant molecule in a non-targeting carrier to administer to an animal treat a metastatic cancer, is from about 100 μg to about 4 mg of total recombinant molecules,

15 more preferably from about 150 μg to about 3 mg of total recombinant molecules, and even more preferably from about 200 μg to about 2 mg of total recombinant molecules. A preferred single dose of a superantigen-encoding recombinant molecule complexed with liposomes to administer

20 to an animal to treat a metastatic cancer, is from about 100 μg of total recombinant molecules per 800 nmol of liposome to about 4 mg of total recombinant molecules per 32 μmol of liposome, more preferably from about 200 μg per 1.6 μm of liposome to about 3 mg of total recombinant

25 molecules per 24 μmol of liposome, and even more preferably from about 400 μg per 3.2 μmol of liposome to about 2 mg of total recombinant molecules per 16 μmol of liposome.

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A preferred single dose of a cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule in a non-targeting carrier to administer to an animal to treat a metastatic cancer, is from about 100 μg

5   to about 4.0 mg of total recombinant molecules, more preferably from about 150 μg to about 3 mg of total recombinant molecules, and even more preferably from about 200 μg to about 2 mg of total recombinant molecules. A preferred single dose of a cytokine-encoding recombinant

10   molecule or a chemokine-encoding recombinant molecule complexed with liposomes to administer to an animal to treat a metastatic cancer, is from about 100 μg of total recombinant molecules per 800 nmol of liposome to about 4.0 mg of total recombinant molecules per 32 μmol of liposome,

15   more preferably from about 200 μg per 1.6 μmol of liposome to about 3 mg of total recombinant molecules per 24 μmol of liposome, and even more preferably from about 400 μg per 3.2 μmol of liposome to about 2 μg of total recombinant molecules per 16 μmol of liposome.

20   According to the present invention, a single dose of a therapeutic composition useful to treat a lesion, comprising a superantigen-encoding recombinant molecule in a non-targeting carrier or liposomes, respectively, and a cytokine-encoding recombinant molecule in a non-targeting

25   carrier or liposomes, respectively, is substantially similar to those doses used to treat a tumor (as described in detail above).

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The number of doses administered to an animal is dependent upon the extent of the disease and the response of an individual patient to the treatment. For example, a large tumor may require more doses than a smaller tumor. In some cases, however, a patient having a large tumor may require fewer doses than a patient with a smaller tumor, if the patient with the large tumor responds more favorably to the therapeutic composition than the patient with the smaller tumor. Thus, it is within the scope of the present invention that a suitable number of doses includes any number required to cause regression of a disease. A preferred protocol is monthly administrations of single doses (as described above) for up to about 1 year. A preferred number of doses of a therapeutic composition comprising a superantigen-encoding recombinant molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule in a non-targeting carrier or complexed with liposomes in order to treat a tumor is from about 1 to about 10 administrations per patient, preferably from about 2 to about 8 administrations per patient, and even more preferably from about 3 to about 5 administrations per patient. Preferably, such administrations are given once every 2 weeks until signs of remission appear, then once a month until the disease is gone.

A preferred number of doses of a therapeutic composition comprising a superantigen-encoding recombinant

molecule; or a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule in a non-targeting carrier or complexed with liposomes in

5   order to treat a metastatic cancer, is from about 2 to about 10 administrations patient, more preferably from about 3 to about 8 administrations per patient, and even more preferably from about 3 to about 7 administrations per patient. Preferably, such administrations are given once

10   every 2 weeks until signs of remission appear, then once a month until the disease is gone.

According to the present invention, the number of doses of a therapeutic composition to treat a lesion comprising a superantigen-encoding recombinant molecule; or

15   a combination of a superantigen-encoding recombinant molecule, with a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule, in a non-targeting carrier or liposomes, respectively, is substantially similar to those number of doses used to

20   treat a tumor (as described in detail above).

A therapeutic composition is administered to an animal in a fashion to enable expression of an introduced recombinant molecule of the present invention into a curative protein in the animal to be treated for disease.

25   A therapeutic composition can be administered to an animal in a variety of methods including, but not limited to, local administration of the composition into a site in an animal. Examples of such sites include lymph nodes, a site

that contains abnormal cells or pathogens to be destroyed (e.g., injection locally within the area of a tumor or a lesion); and systemic administration.

Therapeutic compositions to be delivered by local
5   administration include: (a) recombinant molecules of the present invention in a non-targeting carrier (e.g., as "naked" DNA molecules, such as is taught, for example in Wolff et al., 1990, *Science 247*, 1465-1468); and (b) recombinant molecules of the present invention complexed to
10 a delivery vehicle of the present invention. Suitable delivery vehicles for local administration comprise liposomes. Delivery vehicles for local administration can further comprise ligands for targeting the vehicle to a particular site (as described in detail herein).

15   A preferred method of local administration is by direct injection. Direct injection techniques are particularly useful for the treatment of disease by, for example, injecting the composition into a mass formed by abnormal cells, a lymph node or a granuloma mass induced by
20 pathogens. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of a tumor mass, a lymph node, a granuloma mass or a cancer cell. Administration of a composition locally
25 within an area of a mass or a cell refers to injecting the composition centimeters and preferably, millimeters within the mass or the cell. A preferred tumor mass to inject includes discrete inner body and cutaneous solid tumors.

A preferred inner body tumor to inject includes a discrete solid tumor that forms in the brain, breast, liver, kidney, colon, prostate, testicular, ovary, spleen and/or lymph node. A preferred cutaneous tumor to inject includes a

5   discrete solid melanoma.

A preferred lymph node to inject includes a draining lymph node that "drains" a site containing abnormal cells or pathogens. As used herein, the term "draining lymph node" refers to a lymph node that is located downstream of

10   a site containing abnormal cells or pathogens is based on the direction of the lymphatic flow of an animal (see general discussion in Hole, *Human Anatomy and Physiology*, Edward G. Jaffe, ed., Wm. C Brown Publishers, Dubuque, IA; and G.C. Christiansen et al., *Anatomy of the Dog*, W.B.

15   Saunders Publishers, Philadelphia, PN, 1979; both of which are incorporated herein by this reference). A preferred draining lymph node to inject comprises the draining lymph node most proximal to a site containing abnormal cells or pathogens. Thus, a skilled artisan can choose the site of

20   lymph node injection based upon the location of the site containing abnormal cells or pathogens. Examples of lymph nodes to injection include: the mandibular lymph node if a tumor is located in the oral cavity; and the superficial cervical lymph node of a tumor is located in the front leg

25   region. Effector cells travel from a site containing abnormal cells or pathogens. Injection of a therapeutic composition of the present invention into a lymph node can result in expression of a superantigen, a cytokine and/or

a chemokine by an effector cell from the lymph node or that has drained into the lymph node. Such expression can result in the activation of T lymphocytes, which can travel back to the site containing abnormal cells or pathogens to

5 enhance the immune response at the site.

Another method of local administration is to contact a therapeutic composition of the present invention in or around a surgical wound. For example, a patient can undergo surgery to remove a tumor. Upon removal of the tumor, the

10 therapeutic composition can be coated on the surface of tissue inside the wound or the composition can be injected into areas of tissue inside the wound. Such local administration is useful for treating cancer cells not excised by the surgical procedure, as well as, preventing

15 recurrence of the primary tumor or development of a secondary tumor in the area of the surgery.

In one embodiment, a therapeutic composition of the present invention can be introduced to a tumor cell *in vivo*. In another embodiment, a therapeutic composition of

20 the present invention can be introduced to a non-tumor cell *in vivo* or *in vitro*. Methods to introduce a therapeutic composition *in vivo* are disclosed herein. Methods to introduce a therapeutic composition *in vitro* include methods standard in the art, such as culturing cells in the

25 presence of a therapeutic composition for a sufficient amount of time to enable a nucleic acid molecule of the present invention to pass through the plasma membrane in a cell and subsequently to be expressed in the cell.

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Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site, preferably ligands for targeting the vehicle to a site of a cancer or a lesion (depending upon the disease being treated). For cancer treatment, ligands capable of selectively binding to a cancer cell or to a cell within the area of a cancer cell are preferred. Systemic administration is useful for the treatment of both tumor and metastatic cancer and systemic infectious diseases. Systemic administration is particularly useful for the treatment of metastatic forms of cancer, in which the cancer cells are dispersed (i.e., not localized within a single tumor mass). Systemic administration is particularly advantageous when organs, in particular difficult to reach organs (e.g., heart, spleen, lung or liver) are the targeted sites of treatment.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., *Proc. Natl. Acad. Sci. USA* 189:11277-11281, 1992, which is incorporated herein by reference in its entirety). Oral delivery can be

performed by complexing a therapeutic composition of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or

5   tablets, such as those known in the art. Topical delivery can be performed by mixing a therapeutic composition of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Therapeutic compositions of the present invention can

10   be administered to any animal, preferably to mammals and birds, and more preferably to humans, house pets, economic produce animals and zoo animals. Economic produce animals include animals to be consumed or that produce useful products (e.g., sheep for wool production). Zoo animals

15   include those animals harbored in zoos. Preferred animals to protect include humans, dogs, cats, sheep, cattle, horses and pigs, with humans and dogs being particularly preferred. While a therapeutic composition of the present invention is effective to treat disease in inbred species

20   of animals, the composition is particularly useful for treating outbred species of animals, in particular those having tumors.

Yet another embodiment of the present invention is a method to suppress T cell activity in an animal, the method

25   comprising administering to an animal an effective amount of a therapeutic composition comprising: (a) a naked nucleic acid molecule encoding a superantigen; and (b) a pharmaceutically acceptable carrier, in which the nucleic

acid molecule is operatively linked to a transcription
control sequence, and in which the therapeutic composition
is targeted to a site in the animal that contains excessive
T cell activity.

5   Suitable embodiments, single dose sizes, number of
doses and modes of administration of a therapeutic
composition of the present invention useful in a treatment
method of the present invention are disclosed in detail
herein.

10   A therapeutic composition of the present invention is
also advantageous for the treatment of autoimmune diseases
in that the composition suppresses the harmful stimulation
of T cells by autoantigens (i.e., a "self", rather than a
foreign antigen).   Superantigen-encoding recombinant
15 molecules in a therapeutic composition, upon transfection
into a cell, produce superantigens that delete harmful
populations of T cells involved in an autoimmune disease.
A preferred therapeutic composition for use in the
treatment of autoimmune disease comprises a superantigen-
20 encoding recombinant molecule of the present invention. A
more preferred therapeutic composition for use in the
treatment of autoimmune disease comprises a superantigen-
encoding recombinant molecule combined with a non-targeting
carrier of the present invention, preferably saline or
25 phosphate buffered saline.   Such   a   therapeutic
composition of the present invention is particularly useful
for the treatment of autoimmune diseases, including but not
limited   to,   multiple   sclerosis,   systemic   lupus

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erythematosus, myasthenia gravis, rheumatoid arthritis, insulin dependent diabetes mellitus, psoriasis, polyarteritis, immune mediated vasculitides, immune mediated glomerulonephritis, inflammatory neuropathies and

5 sarcoidosis.

A single dose of a superantigen-encoding nucleic acid molecule in a non-targeting carrier to administer to an animal to treat an autoimmune disease is from about 0.1 μg to about 200 μg of total recombinant molecules per kilogram

10 (kg) of body weight, more preferably from about 0.5 μg to about 150 μg of total recombinant molecules per kg of body weight, and even more preferably from about 1 μg to about 10 μg of total recombinant molecules per kg of body weight.

The number of doses of a superantigen-encoding

15 recombinant molecule in a non-targeting carrier to be administered to an animal to treat an autoimmune disease is an injection about once every 6 months, more preferably about once every 3 months, and even more preferably about once a month.

20 A preferred method to administer a therapeutic composition of the present invention to treat an autoimmune disease is by local administration, preferably direct injection. Direct injection techniques are particularly important in the treatment of an autoimmune disease.

25 Preferably, a therapeutic composition is injected directly into muscle cells in a patient, which results in prolonged expression (e.g., weeks to months) of a recombinant molecule of the present invention. Preferably, a

recombinant molecule of the present invention in the form of "naked DNA" is administered by direct injection into muscle cells in a patient.

Another aspect of the present invention is an adjuvant for use with a nucleic acid-based vaccine to protect an animal from a disease or a remedy to treat a diseased animal. Adjuvants of the present invention comprise: (a) a superantigen-encoding nucleic acid molecule of the present invention; or (b) a combination of a superantigen-encoding nucleic acid molecule of the present invention with a cytokine nucleic acid molecule of the present invention, a chemokine nucleic acid molecule of the present invention or mixtures thereof.

Suitable compounds to combine with an adjuvant of the present invention, to form an adjuvant composition (i.e., a vaccine composition useful as a preventative therapeutic reagent or a therapeutic remedy useful to alleviate a disease) of the present invention, include any compound that is administered to an animal as an immunogen. As used herein, an immunogen of the present invention comprises a compound capable of eliciting an immune response in an animal. Preferably, an immunogen of the present invention is derived from a foreign agent including a pathogen. Also preferably, an immunogen of the present invention includes an allergen (organic or inorganic), tumor antigens and self-antigens.

A preferred immunogen is derived from a pathogen including, but not limited to, a virus, a bacteria, a

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eukaryotic parasite and unicellular protozoa (e.g., amoeba). Preferred eukaryotic parasites include protozoan parasites, helminth parasites (such as nematodes, cestodes, trematodes, ectoparasites and fungi.

5   A preferred immunogen also includes an allergen including, but not limited to, a plant allergen, an animal allergen, a bacterial allergen, a parasitic allergen, a metal-based allergen that causes contact sensitivity and inorganic allergens such as silica, beryllium, xenobiotics,

10   synthetic drugs and dyes. A more preferred allergen includes weed, grass, tree, peanut, mite, flea, cat, house dust and bacterial products antigens.

        A preferred immunogen derived from a bacteria includes an immunogen that protects an animal from or alleviates

15   *Mycobacterium* infection, in particular *M. tuberculosis*, *M. leprae*, *M. avium*, and/or *M. bovis* infection. A more preferred bacterial immunogen of the present invention includes a peptide, mimetopes thereof and compositions containing the same, as disclosed in U.S. Patent Serial No.

20   08/484,169, filed June 7, 1995, which is incorporated herein by this reference. In one embodiment, an immunogen comprises a nucleic acid molecule encoding an immunogenic protein. Such immunogen-encoding nucleic acid molecules can be designed by those of skill in the art

25   based upon the amino acid sequence of the immunogen. In addition, a recombinant molecule encoding an immunogen of the present invention can be produced using the recombinant DNA technology disclosed herein and known to those of skill

in the art. In other embodiments, an immunogen can comprise a peptide, a polypeptide or a chemical compound as disclosed herein. All such embodiments of an immunogen are useful with an adjuvant of the present invention.

5   In order to treat an animal (i.e., vaccinate or remedy), an adjuvant composition of the present invention is administered to the animal in an effective manner such that the composition is capable of protecting an animal from or alleviating a disease. For example, an adjuvant, 10   when administered to an animal in an effective manner, is able to stimulate effector cell immunity in a manner that is sufficient to prevent an initial or continued disease response by the subject animal.

An effective administration protocol (i.e., 15   administering an adjuvant composition in an effective manner) comprises suitable dose parameters, and modes and times of administration that result in the treatment of an animal. Effective dose parameters and modes of administration can be determined using methods standard in 20   the art for a particular adjuvant composition. Such methods include, for example: determination of side effects (i.e., toxicity) of an adjuvant composition; progression of a disease upon administration of an adjuvant composition; magnitude and/or duration of antibody response by an animal 25   against an immunogen contained in an adjuvant composition; magnitude and/or duration of a cell mediated immune response in an animal against an adjuvant composition; similarity of an immune response to an adjuvant composition

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in different species of animals; and/or effect of breed (in non-human animals) or race (in humans) on responsiveness to an adjuvant composition. In particular, the effectiveness of dose parameters and modes of administration of an

5   adjuvant composition of the present invention can be determined by assessing antibody production *in vivo*, skin test sensitivities *in vivo*, cytokine production *in vitro*, antigen-specific proliferation *in vitro*, cytotoxic T cell activity *in vitro*, reduction of tumor burden *in vivo* and/or

10 reduction of infectious agent burden *in vivo*. Tests standard in the art can be used to determine antibody production (e.g., enzyme-linked immunoassays), skin test sensitivities (e.g., subcutaneous injection of an immunogen into a vaccinated animal to assess weal formation,

15 induration and erythema), cytokine production (e.g., immunoassays using cytokine-specific antibodies or bio- assays using cytokine-dependent cell lines), antigen- specific proliferation (e.g., ^{3}H-thymidine incorporation), cytotoxic T cell activity (e.g., measure release of ^{51}Cr

20 from target cells), reduction of tumor burden (e.g., measure size of a tumor) and/or reduction of infectious agent burden (e.g., obtaining, for example, viral titers, bacterial colony counts or parasite counts).

An effective dose refers to a dose capable of

25 immunizing an animal against an immunogen. Effective doses can vary depending upon, for example, the adjuvant used, the immunogen being administered, and the size and type of the recipient animal. Effective doses to treat an animal

to an immunogen include doses administered over time that are capable of preventing or alleviating a disease in an animal to, for example, a pathogen or allergen. For example, a first treatment dose can comprise an amount of

5   an adjuvant composition of the present invention that causes a minimal hypersensitive response when administered to a hypersensitive animal. A second treatment dose can comprise a greater amount of the same adjuvant composition than the first dose. Effective treatment doses can comprise

10   increasing concentrations of the adjuvant composition necessary to treat an animal such that the animal does not exhibit signs of a disease.

In accordance with the present invention, a suitable single dose is a dose that is capable of vaccinating an

15   animal against a foreign agent when administered one or more times over a suitable time period. For example, a preferred single dose of an adjuvant composition of the present invention is from about 100 μg to about 1 mg of the adjuvant composition per kilogram body weight of the

20   animal. Further treatments with the adjuvant composition can be administered from about 1 week to about 1 year after the original administration. Further treatments with the adjuvant composition preferably are administered when the animal is no longer protected from an immunogen to which

25   the animal has been treated. Particular administration doses and schedules can be developed by one of skill in the art based upon the parameters discussed above.

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The number of doses administered to an animal is dependent upon the immunogen and the response of an individual patient to the adjuvant composition. For example, treatment of one strain of virus may require more

5   doses than treatment of a more immunogenic strain of virus. Thus, it is within the scope of the present invention that a suitable number of doses includes any number required to treat an animal. A preferred number of doses of an adjuvant composition comprising a superantigen-encoding recombinant

10   molecule, and/or a cytokine-encoding recombinant molecule and/or a chemokine-encoding recombinant molecule is from about 2 to about 20 administrations, preferably from about 3 to about 10 administrations, and even more preferably from about 3 to about 5 administrations per patient per

15   year. Preferably, such administrations are given once every 2 weeks until, for example, antibody production against an immunogen increases or decreases, cell mediated immunity increases, and/or a clinical response is observed when an adjuvant composition is administered as a therapeutic

20   remedy.

A preferred single dose of the superantigen-encoding recombinant molecule is an amount that, when transfected into a muscle cells, skin tissue, lung cells or other suitable cellular sites, leads to the production of from

25   about 10 femtograms (fg) to about .01 μg, preferably from about 100 fg to about 1 picogram (pg), and more preferably from about 1 pg to about 5 pg of superantigen per transfected cell. A preferred single dose of a cytokine-

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encoding recombinant molecule is an amount that when transfected into a target cell population leads to the production of from about 10 pg to about .01 µg, preferably from about 100 fg to about 2 pg, and more preferably about

5   1 pg of cytokine per transfected. A preferred single dose of a chemokine-encoding recombinant molecule is an amount that when transfected into a target cell population leads to the production of from about 1 pg to about .01 µg, preferably from about 0.1 pg to about 10 pg, and more

10   preferably about 1 pg of chemokine per transfected.

In one embodiment, an adjuvant composition of the present invention comprises up to about 50% of an immunogen-encoding recombinant molecule and up to about 50% of a superantigen-encoding recombinant molecule.

15   Preferably, an adjuvant composition of the present invention comprises no more than about 1.5 mg of immunogen-encoding recombinant molecule and no more than about 1.5 mg of superantigen-encoding recombinant molecule, more preferably no more than about 1 mg of immunogen-encoding

20   recombinant molecule and no more than about 1 mg of superantigen-encoding recombinant molecule, and even more preferably no more than about 0.5 mg of immunogen-encoding recombinant molecule and no more than about 0.5 mg of superantigen-encoding recombinant molecule per animal.

25   In another embodiment, an adjuvant composition of the present invention comprises an immunogen-encoding recombinant molecule in an amount up to about 66% by weight of the composition and a superantigen-encoding recombinant

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molecule in an amount up to about 33% by weight of the composition. Preferably, an adjuvant composition of the present invention comprises no more than about 2000 μg of immunogen-encoding recombinant molecule and no more than

5   about 1000 μg of superantigen-encoding recombinant molecule, more preferably no more than about 1400 μg of immunogen-encoding recombinant molecule and no more than about 660 μg of superantigen-encoding recombinant molecule, and even more preferably no more than about 670 μg of

10 immunogen-encoding recombinant molecule and no more than about 330 μg of superantigen-encoding recombinant molecule per animal.

In another embodiment, an adjuvant composition of the present invention comprises an immunogen-encoding

15 recombinant molecule in an amount up to about 50% of the composition; a superantigen-encoding recombinant molecule in an amount up to about 25% of the composition; and a cytokine-encoding recombinant molecule or chemokine-encoding recombinant molecule or mixtures thereof, in an

20 amount up to about 25% of the composition. According to the present embodiment, a cytokine-encoding recombinant molecule or a chemokine-encoding recombinant molecule can be used alone or in combination with each other. When used in combination, the ratio of cytokine-encoding recombinant

25 molecule to chemokine-encoding recombinant molecule can be varied according to need. The ratio can be determined based upon the effectiveness of the adjuvant composition at

vaccinating an animal against a foreign agent using the methods and parameters disclosed herein.

In one embodiment, an adjuvant composition of the present invention comprises: no more than about 2000 μg of immunogen-encoding recombinant molecule, no more than about 500 μg of superantigen-encoding recombinant molecule, and no more than about 500 μg of cytokine-encoding recombinant molecule or no more than about 500 μg of chemokine-encoding recombinant molecule; more preferably no more than about 1400 μg of immunogen-encoding recombinant molecule, no more than about 300 μg of superantigen-encoding recombinant molecule, and no more than about 300 μg of cytokine-encoding recombinant molecule or no more than about 300 μg of chemokine-encoding recombinant molecule; and even more preferably no more than about 660 μg of immunogen-encoding recombinant molecule, no more than about 160 μg of superantigen-encoding recombinant molecule, and no more than about 160 μg of cytokine-encoding recombinant molecule or no more than about 160 μg of chemokine-encoding recombinant molecule per animal.

In another embodiment, an adjuvant composition of the present invention comprises: no more than about 2000 μg of immunogen-encoding recombinant molecule, no more than about 500 μg of superantigen-encoding recombinant molecule, and no more than about 250 μg of cytokine-encoding recombinant molecule and no more than about 250 μg of chemokine-encoding recombinant molecule; more preferably no more than about 1000 μg of immunogen-encoding recombinant molecule,

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no more than about 250 μg of superantigen-encoding recombinant molecule, and no more than about 125 μg of cytokine-encoding recombinant molecule and no more than about 125 μg of chemokine-encoding recombinant molecule;

5   and even more preferably no more than about 660 μg of immunogen-encoding recombinant molecule, no more than about 160 μg of superantigen-encoding recombinant molecule, and no more than about 80 μg of cytokine-encoding recombinant molecule and no more than about 80 μg of chemokine-encoding

10   recombinant molecule per animal.

Adjuvant compositions are preferably delivered by intramuscular administration in the form of "naked" DNA molecules, such as disclosed herein. Preferably, an adjuvant composition of the present invention is delivered

15   by intramuscular, intravenous, intraperitoneal and/or intraarterial injection, and/or injection directly into specific cellular locations (e.g., into a tumor). Preferred sites of intramuscular injections include caudal thigh muscle, back muscle and into the buttocks of a human.

20

Preferably, an adjuvant composition of the present invention comprises a suitable pharmaceutically acceptable carrier for delivering the composition intramuscularly. A preferred carrier for use with an adjuvant includes

25   phosphate buffered saline, water, Ringer's solution, dextrose solution, Hank's balanced salt solution and normal saline. A more preferred carrier includes phosphate

buffered saline and normal saline, with phosphate buffered saline being even more preferred.

Preferably, an adjuvant composition of the present invention comprises a mixture including a superantigen

5   encoding molecule including an SEA-encoding recombinant molecule, an SEB-encoding recombinant molecule or mixtures thereof, and an immunogen-encoding recombinant molecule of the present invention; a superantigen encoding molecule including an SEA-encoding recombinant molecule, an SEB-

10 encoding recombinant molecule or mixtures thereof, a cytokine encoding molecule including a GM-CSF-encoding recombinant molecule and an immunogen-encoding recombinant molecule of the present invention; or a superantigen encoding molecule including an SEA-encoding recombinant

15 molecule, an SEB-encoding recombinant molecule or mixtures thereof, a chemokine encoding molecule including a MIP1α, MIP1β, IL-8 or RANTES recombinant molecule and an immunogen-encoding recombinant molecule of the present invention.

20   In a preferred embodiment, an adjuvant of the present invention includes the following recombinant molecules contained in phosphate buffered saline: (1) PCR_3-SEA, PCR_3-SEA.S, PCR_3-SEB, PCR_3-SEB.S, PCR_3-TSST and mixtures thereof; (2) mixtures of up to about 50% PCR_3-SEA, PCR_3-SEA.S, PCR_3-

25 SEB, PCR_3-SEB.S and/or PCR_3-TSST, and up to about 50% PCR_3-GM_3; (3)

mixtures of up to about 50% PCR_3-SEA, PCR_3-SEA.S, PCR_3-SEB, PCR_3-SEB.S and/or PCR_3-TSST, and up to about 50% PCR_3-MIP1α;

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(4) mixtures of up to about 50% PCR$_3$-SEA, PCR$_3$-SEA.S, PCR$_3$-SEB, PCR$_3$-SEB.S and/or PCR$_3$-TSST, and up to about 50% PCR$_3$-MIP1β; (5) mixtures of up to about 50% PCR$_3$-SEA, PCR$_3$-SEA.S, PCR$_3$-SEB, PCR$_3$-SEB.S and/or PCR$_3$-TSST, and up to about 50%

5   PCR$_3$-RANTES; (6) mixtures of up to about 50% PCR$_3$-SEA, PCR$_3$-SEA.S, PCR$_3$-SEB, PCR$_3$-SEB.S and/or PCR$_3$-TSST, up to about 25% PCR$_3$-GM$_3$, and up to about 25% PCR$_3$-MIP1α, PCR$_3$-MIP1β and/or PCR$_3$-RANTES.

According to the present invention, a preferred

10   embodiment of an adjuvant composition of the present invention includes: (1) an immunogen-encoding recombinant molecule the present invention in an amount up to about 50% of the composition and a preferred embodiment of an adjuvant of the present invention in an amount up to about

15   50% of the composition; or (2) an immunogen-encoding recombinant molecule in an amount up to about 66% of the composition and a preferred embodiment of an adjuvant of the present invention in an amount up to about 33% of the composition, in phosphate buffered saline.

20   The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

Example 1

25   This example describes the production of recombinant molecules encoding superantigens and cytokines.

Full-length cDNA encoding *Staphylococcal enterotoxin B* (SEB; SEQ ID NO:1) and *Staphylococcal enterotoxin A* (SEA;

SEQ ID NO:3) were produced by polymerase chain reaction (PCR) amplification using templates obtained from Dr. John Kappler (National Jewish Center for Immunology and Respiratory Disease, Denver, CO). A truncated form of SEB

5 lacking the leader sequence, which spans base pairs 46 to 773 (referred to herein as SEB.S), was prepared by PCR amplification using the primers 5' GGGAATTCCATGGAGAGTCAACCAG 3' (SEQ ID NO:7) and 3' GCGGATCCTCACTTTTTCTTTGT 5' (SEQ ID NO:8). A truncated form

10 of SEA lacking the signal sequence, which spans base pairs 46 to 751 (referred to herein as SEA.S), was prepared by PCR amplification using the primers 5' GGGAATTCCATGGAGAGTCAACCAG 3' (SEQ ID NO:9) and 5' GCAAGCTTAACTTGTATATAAATAG 3' (SEQ ID NO:10). Full-length

15 cDNA encoding Toxic Shock Syndrome Toxin (TSST; SEQ ID NO:5) was produced by PCR amplification using a template obtained from Dr. Brian Kotzin (National Jewish Center for Immunology and Respiratory Disease, Denver, CO), using the primers:

20 5' CGGGGTACCCCGAAGGAGGAAAAAAAAATGTCTACAAACGATAATATAAAG 3' (SEQ ID NO:11); and

3' TGCTCTAGAGCATTAATTAATTTCTGCTTCTATAGTTTTTAT 5' (SEQ ID NO:12).

Each cDNA clone was ligated into the eukaryotic

25 expression vector PCR_3 (In Vitrogen, San Diego, CA) using standard cloning methods. The full-length SEB cDNA cloned into PCR_3 is referred to herein as PCR_3-SEB; the full-length SEA cDNA cloned into PCR_3 is referred to herein as PCR_3-SEA;

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the full-length TSST cDNA cloned into PCR₃ is referred to herein as PCR₃-TSST; the truncated SEB cDNA cloned into PCR₃ is referred to herein as PCR₃-SEB.S; and the truncated SEA cDNA cloned into PCR₃ is referred to herein as PCR₃-SEA.S.

5   A cDNA for canine GM-CSF was produced by PCR amplification of total RNA extracted from Concavalin A-stimulated normal canine peripheral blood mononuclear cells (PBMC) using canine GM-CSF primers designed based on the published canine GM-CSF cDNA (Nash, *ibid*.). The total RNA

10   was reverse transcribed using the reverse transcriptase enzyme and oligoT primers. The canine GM-CSF cDNA was then amplified using PCR and specific 5' and 3' primers. The PCR product was cloned into the PCR₃ vector, the resulting recombinant molecule is referred to herein as PCR₃-GM₃.

15   <u>Example 2</u>

This example describes the expression of DNA encoding superantigens in mammalian CHO cells following transfection.

Isolated PCR₃-SEB.S, PCR₃-SEA.S and PCR₃-TSST were

20   transformed into *E. coli* cells and ampicillin-resistant bacterial colonies were screened for the presence of the plasmid. Selected colonies were then cultured in large scale culture (liter volume). Plasmid DNA was isolated using standard methods. A typical plasmid yield was 20 mg

25   plasmid DNA from one liter of bacteria-containing culture medium. Plasmid DNA was transfected into Chinese hamster ovary cells (CHO) by lipofection (Lipofectamine, Gibco-BRL, Gaithersburg, MD) using methods provided by the

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manufacturer. About 2.0 μg of each plasmid DNA was separately transfected into about 10^6 CHO cells.

The transfected CHO cells were cultured for 48 hours. Supernatants and cell lysates were then isolated to
5   determine the amount of intracellular and secreted SAg protein produced by the transfected cells. Cell lysates were prepared by detaching and sonicating the transfected cells to prepare cell lysates to measure activity. SAg protein activity in each sample was measured by
10 quantitating the ability of the SAg protein to stimulate lymphocyte contained in a PBMC population using the following method. Supernatants and lysates to be tested were added in serial dilutions to triplicate wells of a 96-well plate containing 5 X 10^5 PBMC in a total volume of 200
15 μl per well. After 3 days, the wells were pulsed with ^{3}H thymidine and incubated for 18 hours. The radioactivity incorporated into the PBMC's were quantitated on a beta counter. Negative controls included CHO cells transfected with the DNA vector without an inserted gene (mock) and
20 positive controls were purified recombinant SAg proteins.

The results were plotted as the mean incorporated thymidine in counts per minute and are shown in Fig. 1. The results indicate that both supernatants and lysates of
25 CHO cells transfected with PCR$_3$-SEB.S, PCR$_3$-SEA.S and PCR$_3$-TSST stimulated strong proliferation of the PBMC's, compared to mock transfected cultures. The activity in supernatants in some cases exceeded that in cell lysates.

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Thus, DNA encoding bacterial SAg proteins are capable of being transcribed and translated in mammalian cells in biologically active form. The results also indicate that the amounts of biologically active SAg protein are active

5   produced by the transfected cells was sufficient to stimulate T cell proliferation.

Example 3

This example describes the expression of DNA encoding superantigens in canine melanoma cells following

10   transfection.

A melanoma cell line was established from an oral malignant melanoma obtained by biopsy from a canine patient by isolating a portion of a melanoma tumor, digesting that portion with collagenase and plating the released cells in

15   24 well plates using Iscove Modified Dulbecco's Medium (IMDM) with 10% fetal calf serum. Melanoma cells were transfected with PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST by lipofection as described in Example 2. The cells were then irradiated (15,000 Rads). Four samples of each sample of

20   transfected melanoma cells were prepared, in which decreasing numbers of the transfected cells were added to normal canine PBMC (5 X 10^5/well). Each sample was prepared in triplicate in a 96 well plate. After 3 days, proliferation was quantitated as described in Example 2.

25   Non-transfected melanoma cells were used as negative control samples. The results were plotted as the mean incorporated thymidine in counts per minute and are shown in Fig. 2. The results indicate that Canine PBMC

proliferated when cultured with canine melanoma cells transfected with PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST, exhibiting a dose-dependent increase in proliferation as increasing numbers of irradiated tumor cells were used.

5   Thus, melanoma tumor cells can be transfected and can express biologically active SAg protein. The results also show that the transfected melanoma cells continue to release biologically active SAg protein after irradiation, indicating that transfected tumor cells would also be

10 useful as an autologous tumor vaccine as described in detail in the present application.

Example 4

This example describes the long term expression of DNA encoding SEB.S and SEA.S in stably transfected CHO cells.

15   To determine whether the SAg protein activity detected in supernatants of transfected CHO cells (described in Example 2) represented actual secretion or simple release from dying cells, stably transfected CHO lines were prepared using either PCR_3-SEB.S, PCR_3-SEA.S or vector with

20 no cDNA insert (control). About 2 x 10^6 CHO cells were transfected with about 2 μg of plasmid DNA by lipofection. The transfected cells were then cultured in G418 (1 mg/ml) for 3 weeks to obtain stable transfectants. The G418 selected CHO cells were seeded into 9 individual tissue

25 culture wells, allowed to adhere for 4 hours, and then fresh tissue culture media was added. Supernatants were harvested sequentially, beginning at time zero and continuing for 36 hours. Supernatants were added to PBMC

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to assay for SAg protein activity, as described in Example 2.

The results were plotted as the mean proliferation stimulating activity contained in supernatants at each time
5   point and are shown in Figs. 3A and 3B. The results indicate that a steady time-dependent increase in PBMC stimulatory activity was observed in supernatants from CHO cells stably transfected with both PCR_3-SEB.S and PCR_3-SEA.S. Thus, transfection of mammalian cells with PCR_3-
10   SEB.S, PCR_3-SEA.S results in long term expression of biologically active SAg protein. The data indicates that transfected mammalian cells can serve as a sustained source of SAg protein production.

Example 5

15   This example describes that transfection of PCR_3-SEA.S DNA in melanoma cells results in the expression of biologically active SEA.S protein.

Superantigens are capable of stimulating the proliferation of T cells bearing certain $V\beta$ domains in
20   their T cell receptor (TCR). SEA is known to stimulate T cells having a $V\beta3+$ TCR in mice. SEB does not stimulate $V\beta3+$ T cells. Therefore, an experiment was performed to assess the ability of SEA.S protein expressed by melanoma cells transfected with PCR_3-SEA.S DNA to stimulate the
25   proliferation of a T cell clone (AD10) expressing the $V\beta3+$TCR.

B16 melanoma cells were transfected with PCR_3-SEA.S DNA, PCR_3-SEB.S or PCR_3 vector DNA with no insert (mock).

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The cells were then irradiated (18,000 Rads) and plated in triplicate in a 96 well plate at a concentration of about 1×10^4 per well. About 1×10^5 AD10 cells were added to each well. Next, irradiated syngeneic spleen cells were

5   added to each well as a source of antigen presenting cells for the superantigen and T cell interaction. Negative controls included mock transfected cells; positive controls included recombinant SEA (10ng/ml). The cells were incubated for 48 hours. ^{3}H thymidine was then added to each

10 well and the proliferative response quantitated.

The results were plotted as the mean incorporated thymidine in counts per minute and are shown in Fig. 4. The AD10 cells proliferated strongly in response to SEA.S protein produced by the PCR_3-SEA.S DNA transfected into the

15 B16 cells, with the proliferative response nearly equal to that of the recombinant protein. Thus, the T cell response generated by transfection of melanoma cells with PCR_3-SEA.S DNA is specific for the correct TCR. Cells transfected with PCR_3-SEB.S DNA did not stimulate proliferation of AD10

20 cells, consistent with the predicted TCR specificity of SEA and SEB.

Example 6

This example describes the expression of PCR_3-GM DNA in CHO cells.

25 PCR_3-GM DNA was produced, isolated and transfected into CHO cells using the methods described in Examples 1 and 2. Expression of GM-CSF protein in the CHO cells was measured by the following method. Supernatants were isolated from

the cultures of the transfected cells and non-transfected CHO cells. The supernatants were added to cultures of monocyte cells (obtained from normal canine PBMC) and the ability of the supernatants to support the growth and
5   survival of monocytes was determined. After 4 days in culture with test or control CHO supernatants, monocyte survival was quantitated by addition of methyltetrazolium dye (MTT) that is reduced in viable cells. Absorbance of light at 570 nm (measured using an ELISA reader) is
10   representative of cell survival.

The results are shown in Fig. 5 and indicate that the supernatants from CHO transfected with PCR$_3$-GM DNA stimulated the survival of canine monocytes in culture compared with results obtained using the control
15   supernatants. The level of activity was comparable to that of 1×10^5 units of canine recombinant GM-CSF. Thus, the GM-CSF protein produced by CHO cells transfected with PCR$_3$-GM DNA is biologically active.

Example 7

20   This example demonstrates that the vaccination of mice with autologous tumor cells transfected with PCR$_3$-SEA.S DNA or PCR$_3$-SEB.S DNA induce strong cytotoxic T cell (CTL) activity.

The following experiment studies the ability of non-
25   immunogenic murine melanoma cells (B16 melanoma cells, F10 clone) expressing either PCR$_3$-SEA.S DNA or PCR$_3$-SEB.S to induce CTL responses in mice. B16 cells are known to be non-immunogenic when injected into C57B16/J mice. The

level of CTL responses that can be induced has been shown to correlate with the ability of the immunized animal to reject tumors.

B16 cells were transfected with either PCR$_3$-SEA.S DNA, PCR$_3$-SEB.S or PCR$_3$ DNA lacking insert (mock) using the method described in Example 2. The cells were then irradiated at 12,000 Rads. About 10^6 irradiated cells were then injected subcutaneously into C57B16/J mice. Three weeks later, the mice were sacrificed and their spleen mononuclear cells harvested. Mononuclear cells isolated from the spleen cells were then restimulated *in vitro* with irradiated, non-transfected wild type B16 cells for 6 days in media with interleukin-2 (IL-2). The spleen cells were then added in decreasing numbers to about 5×10^3 of ^{51}Cr-labeled wild type (non-transfected) B16 cells in a standard chromium release assay for CTL activity. After 4 hours, the supernatants were harvested and the percentage of specific lysis of the target B16 melanoma cells was quantitated.

The results are shown in Figs. 6A and 6E and indicate that injection of animals with irradiated transfected melanoma cells induce greater CTL activity than injection with non-transfected cells. This result is consistent with the non-immunogenic nature of B16 cells. Thus, DNA encoding bacterial SAg proteins expressed in transfected tumor cells are capable of eliciting strong CTL-mediated immunity against the non-transfected parental cell. These results suggest that autologous tumor cells transfected

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with DNA encoding a superantigen constitute an effective tumor vaccine for treatment or prevention of metastatic disease.

Example 8

5   This example demonstrates that tumor cells transfected with PCR_3-SEB.S DNA are capable of inducing cytotoxic activity in adjacent T cells.

T cells were prepared from a mouse immunized with non-transfected B16 cells using the methods described in
10   Example 7.   These isolated cells exhibited minimal CTL activity towards non-transfected B16 target cells.   B16 cells were transfected with PCR_3-SEB.S using the methods generally described in Example 2.   Induction of CTL activity by the transfected B16 target cells was assessed
15   in a standard 4 hour chromium release assay as used in Example 7.

The results are shown in Fig. 7 and indicates that B16 cells transfected with PCR_3-SEB.S produced protein that rapidly induced a four-fold increase in CTL activity in T
20   cells that were relatively unresponsive to non-transfected target B16 cells.   Thus, the SEB produced in the vicinity of the isolated T cells by the B16 cells is capable of stimulating such T cells.   The data indicates that tumor cells transfected in vivo with PCR_3-SEB.S can produce
25   biologically active SEB.S that is capable of rapidly activating T lymphocytes in their vicinity and thereby inducing cytotoxic activity against themselves or neighboring tumor cells.

Example 9

This example describes the treatment of canine melanoma with DNA encoding superantigen or GM-CSF.

A.   Criteria for entry and trial design

5   Animals selected for entry into the present study were client owned animals with spontaneous oral malignant melanoma, a highly malignant neoplasm of dogs for which there is no alternative effective treatment. Prior to entry, the owners were required to sign informed consent.

10   The study consisted of an initial 12 week trial response phase with 6 injections given once every 2 weeks, followed by long term once monthly maintenance therapy for those animals that responded during the initial 12 week induction phase. Potential toxicity was assessed by (1) body

15   temperature measured daily for 7 days after injection; (2) physical examination of the injection site; (3) owner's assessment of their pet's attitude and appetite; (4) complete blood counts and biochemistry measurements once monthly. Treatment responses were assessed by: (1)

20   physical measurement of tumor dimensions; (2) tumor photography; (3) thoracic radiographs for metastasis evaluation.

B.   Superantigen + GM-CSF Treatment protocol

DNA samples complexed with liposomes were prepared as

25   follows. PCR₃-SEB.S and PCR₃-GM plasmid DNA prepared from bacterial cultures by the alkaline lysis method, then purified by CsCl banding, were resuspended at a 1.0 mg/ml concentration in sterile PBS. Liposomes were prepared by

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mixing equimolar amounts of N-[1-(33-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA; obtained from Syntex, Corp., Palo Alto, CA) and dioleoyl phosphatidylethanolamine (DOPE; obtained from Avanti Polar Lipids, Birmingham, AL). The

5 lipids were dried in a desiccator and reconstituted at a concentration of 1.0 mg/ml in sterile phosphate buffered saline (PBS), pH 7.0. The reconstituted lipids were sonicated for 5 minutes to produce liposomes having an average size of about 200 nm to about 400 nm.

10 Thirty minutes prior to injection into the patients, the PCR_3-SEB.S and PCR_3-GM DNA was mixed with the liposomes at a ratio of 1.0 μg DNA to 4 nmol liposome, in 1.0 ml sterile PBS. The solution was allowed to complex at room temperature. Two doses of DNA were administered, depending

15 on tumor volume. For tumors less than 3 centimeters (cm) in diameter, 400 μg total DNA (200 μg each of PCR_3-SEB.S and PCR_3-GM DNA) were injected into each tumor. For tumors larger than 3 cm diameter, a total of 800 μg DNA (400 μg each of PCR_3-SEB.S and PCR_3-GM DNA) were injected into each

20 tumor.

For each treatment, the DNA:liposome mixture was injected into the tumor site with a 3 ml syringe and 25 gauge needle. For larger tumors, most of the injection was delivered into tissues at the periphery of the tumor base.

25 For some smaller tumors, injections were also injected directly into tumor tissue. Lymph node tissue having evidence of tumor metastasis was also injected. Injections were performed once every 2 weeks for the first 12 weeks,

then continued twice monthly for those animals in which an
initial treatment response occurred, until complete tumor
regression occurred.   At that time, the frequency of
injections decreased to once monthly.   The toxicity of the

5   treatment was evaluated based on the parameters outlined
above in section A. The results are shown below in Table 1.

Table 1. Patient Log for SEB.S and PCR₃-GM DNA Treatment of Canine Melanoma

Patient	Stage	TN	Tumor Size	Start Date	Response	Comments
Zomax	I	T1bNOMO	1.5 cm diam	5/16/94	CR 51 wks	SEB.S + GM-CSF
Shadow	III	T2bN1bMO	3 cm diam	5/23/94	CR 50 wks	SEB.S + GM-CSF
NG	I	T1NOMO	1.2 cm diam	9/12/94	CR 34 wks	SEB.S + GM-CSF
Maggie	II	T2aNOMO	2 cm diam	8/24/94	PR 33 wks	SEB.S + GM-CSF
K.C.	III	T3aNOMO	> 4 cm diam	10/13/94	SD 12 wk	SEB.S + GM-CSF
Belvedere	III	T2N1bMO	4 cm diam	10/13/94	CR 30 wks	SEB.S + GM-CSF
Nicholas	III	T3bNOMO	> 4 cm diam	2/15/95	SD 12 wks	SEB.S + GM-CSF
Heidi	III	TON1bMO	LN:2cm diam	2/27/95	PR 10 wks	SEB.S + GM-CSF
Bear	III	TON1bMO	LN:2.5cm	4/11/95	SD 4 wks	SEB.S + GM-CSF

Key to terminology in patient data sheets:

Stage: I represents the smallest and III the largest size, with metastases
TNM: World Health Organization staging system
SD   =   stable disease (no tumor growth)
PR   =   partial remission (> 50% decrease in tumor size)
CR   =   tumor completely regressed
PD   =   progressive disease, no response to treatment
MCT =   mast cell tumor
Mammary CA = mammary gland adenocarcinoma (malignant breast cancer)
Thyroid CA = thyroid adenocarcinoma
SCC   = squamous cell carcinoma

The results shown in Table 1 indicate that a treatment response was observed in 6 of 9 dogs treated for the 12 week trial period. This included 4 complete remissions (no residual tumor) and 2 partial remissions (greater than 50%
5   reduction in tumor size). Tumors in the remaining two dogs did not regress, but also did not progress in size during the 12 week trial. On average, a tumor response required 6 to 10 weeks to become apparent. The injections did not cause any inflammation or necrosis at injections sites.
10 Toxicity, either local or systemic, was not observed in any of the 10 patents treated in this study. These results provide evidence of the efficacy of direct DNA injection using DNA encoding superantigen (SEB) and cytokine (GM-CSF) for treatment of spontaneous malignant melanoma in an
15 outbred species.

Canine melanoma is a highly malignant, rapidly growing tumor of dogs, and provides a useful model for the study of treatments for human melanoma. Without treatment, the 50% survival time for animals with stage III disease (5 of the
20 patients in this study) is about 3 months and all animals will be dead by 5 months due to pulmonary metastases. The observation of several long term survivors shown in Table 1 (others have not been treated long enough to evaluate) suggests that the combined DNA immunotherapy approach also
25 has a systemic effect on preventing metastatic diseases.

Another major advantage of this approach is the apparent complete absence of toxicity in the dogs. Since dogs respond to SAg protein similar to humans, it is also

likely that toxicity in humans would also be minimal. The
delivery of DNA encoding superantigens into tumor cells by
transfection and subsequent local expression is sufficient
to induce a strong immune response without inducing
5 toxicity.   Thus   this   genetic   approach   to   tumor
immunotherapy   offers   advantages   over   conventional
chemotherapy and radiation therapy in terms of reducing
patient morbidity. In addition, delivering the SAg protein
by DNA transfection also avoids the potential toxicity
10 associated with systemic administration.

C.   Single Gene Treatment Protocol

To   evaluate   the   effectiveness   of   injecting   DNA
encoding either a superantigen or a cytokine, relative to
combined genetic therapy (SAg-encoding DNA and cytokine-
15 encoding DNA), 2 groups of dogs were treated with either
PCR_3-SEB.S DNA alone (3 dogs) or PCR_3-GM DNA alone (3 dogs;
2 entered, one evaluatable). Similar criteria for entry
and trial design described above in Section A of this
example was applied. Although not formally randomized,
20 after the first 10 dogs were treated with the 2 gene
combination, the next 3 enrollees were assigned the PCR_3-
SEB.S DNA alone group and the next 3 to the PCR_3-GM DNA
alone group. A similar treatment protocol as described
above in section B was applied in this study. Briefly, the
25 DNA was complexed with liposomes and injected once every 2
weeks for the first 12 weeks, then continued twice monthly
for those animals in which an initial treatment response
occurred, until complete tumor regression occurred. The

toxicity of the treatment was evaluated based on the parameters outlined above in section A.   The results are shown below in Table 2.

Table 2. Patient Log for SEB.S or PCR_3-GM DNA alone Treatment of Canine Melanoma

Patient	Stage	TN	Tumor Size	Start Date	Response	Comments
Jessie	II	T2bNOMO	2 cm diam	1/11/95	PD 17 wks	SEB.S alone
Mr. T	III	TON1bMO	LN:2cm diam	2/1/95	PD 14 wks	SEB.S alone
Duffy	II	T2aNOMO	2 cm diam	2/3/95	PD 12 wks	SEB.S alone
Scooter	I	T2aNOMO	2 cm diam	3/24/95	PD 7 wks	GM-CSF alone

5

The results indicated that a tumor response did not occur in any dog receiving PCR₃-SEB.S DNA alone and tumors grew progressively. In addition, one dog (Scooter) treated with PCR₃-GM DNA alone also exhibited progressive growth.

5   These data indicate that treatment with PCR₃-SEB.S DNA alone or PCR₃-GM DNA alone does not induce tumor regression. The data indicate that the marked anti-tumor efficacy of direct DNA injection results from the combined expression of PCR₃-SEB.S DNA and PCR₃-GM DNA in a tumor and adjacent tissues.

10 Example 10

This example describes the treatment of various tumor types with superantigen or GM-CSF encoding DNA.

The efficacy and lack of toxicity of PCR₃-SEB.S DNA and PCR₃-GM DNA was determined for the treatment of dogs with

15 malignancies having similar biological and histological characteristics as human cancers. Dogs with five different cancers (advanced mammary carcinoma, mast cell tumor, thyroid carcinoma, non-oral melanoma, and squamous cell carcinoma) were treated in this study. Animals selected

20 for entry into the present study included dogs with spontaneous malignancies that had received alternative treatments (e.g., chemotherapy and/or surgery) and either, had not responded, or had relapsed.

Therapeutic samples were prepared and injected

25 intratumorally with PCR₃-SEB.S DNA and PCR₃-GM DNA as described above in Example 2. The dogs were treated initially once every 2 weeks for 12 weeks, then continued twice monthly for those animals in which an initial

treatment response occurred. The toxicity of the treatment was evaluated based on the parameters outlined above in Example 9, section A. The results are shown below in Table 3.

Table 3.   Patient Log for SEB.S and PCR₃-GM DNA Treatment of Various Carcinomas

Patient	Tumor Type	Stage	TN	Tumor Size	Start Date	Response	Comments
Emma	Mammary CA	III	T4N1bNMO	1.8 cm diam	8/11/94	PR 22 wks	SEB.S + GM-CSF
Baby	Mammary CA	II	T1aN1bMO	2.6 cm diam	9/12/94	PR 8 wks	SEB.S + GM-CSF
Christa	MCT	IIIa	NA	>2 cm diam	7/27/94	SD 39 wks	SEB.S + GM-CSF
Jack	MCT	IIIa	NA	>3 cm diam	3/28/95	PD 4 wks	SEB.S + GM-CSF
Britt	Thyroid CA	III	T3bNOMO	>7 cm diam	10/14/94	SD 16 wk	SEB.S + GM-CSF
Duncan	Melanoma Toe	NA*	T2N1MO	>4 cm diam	8/11/94	SD 20 wks	SEB.S + GM-CSF
Billy	Melanoma Toe	NA*	TON1bMO	LN 3.5 cm	1/10/95	CR 17 wks	SEB.S + GM-CSF
Scotche	SCC Tonsil	NA	T3NOMO	4 cm diam	3/27/95	SD	SEB.S + GM-CSF

*   Metastases
NA   Not Applicable
CA   Carcinoma
MCT   Mast Cell Tumor
SCC   Squamous Cell Carcinoma

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In this study, toxicity was not observed in any of the animals. Tumor responses (partial remission of the primary tumors) were observed in the animals with mammary carcinoma and neither animal developed additional metastatic disease

5   during the course of the study. Treatment of one dog (Billy) with a large, metastatic (lymph node metastases), non-oral melanoma resulted in complete remission of the cancer. Treatment of the other dog (Duncan) with a large, metastatic (lymph node metastases), non-oral melanoma

10   resulted in prolonged stabilization of the disease. The dog with thyroid cancer (Britt) also experienced prolonged stabilization of the disease with once monthly injections. The response rate for the dogs with mast cell tumors was low. The effectiveness of the treatment on the squamous

15   cell carcinoma is in early stages of evaluation. Taken together, the results indicate that PCR$_3$-SEB.S DNA and PCR$_3$-GM DNA can effectively treat multiple tumor types, in addition to the melanomas reported above in Example 9.

Example 11

20   This example describes the injection of PCR$_3$-SEA.S DNA into muscle cells which induces potent, long-lasting T cell deletion.

Four groups of mice B10.BR (2-3 mice per group) were prepared as follows. Group (1) consisted of untreated mice

25   (control mice). Group (2) consisted of mice injected intraperitoneally with 100 ng of recombinant SEA (rSEA) protein.   Group   (3)   consisted   of   mice   injected

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intramuscularly with 100 µg of PCR$_3$-SEA.S DNA (50 µg per leg, total of 100 µg/mouse). Group (4) consisted of mice injected intramuscularly with 100 µg PCR$_3$ (no insert; mock) DNA (50 µg per leg, total of 100 µg/mouse). The DNA samples

5   were prepared by diluting 100 µl of a solution containing 100 µg of DNA 50:50 (v:v) in sterile PBS prior to injection. The rSEA protein was purified from cultures of *E. coli* cells transformed with the recombinant molecule PKK223 (obtained from Dr. John Kappler) encoding the

10   truncated SEA.S protein lacking a leader sequence.

Beginning 72 hours after injection, mice were tail bled and PBMC prepared for fluorescence activated cell sorter (FACS) analysis. Cells were double labeled with the monoclonal antibodies FITC conjugated-GK1.5 antibody,

15   biotinylated-KJ25 antibody and biotinylated-F23.1, to analyze for expression of CD4, TCR Vβ3 and TCR Vβ8 expression, respectively. The labelled cells were analyzed on an EPICS-C flow cytometer.

The percentage of cells isolated from the experimental

20   mice expressing CD4 that also expressed either Vβ8 or Vβ3 was calculated and compared to percentages expressed by cells isolated from control mice. The mean percentage of CD4+ and Vβ3+ T cells in PBMC was plotted against time after injection. The results are shown in Fig. 8 and

25   indicate that the percentage of CD4+, Vβ3+ T cells declined rapidly in PBMC of mice that received intramuscular injections with PCR$_3$-SEA.S DNA, but not in mice mock

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injected with mock DNA. The percentages of Vβ8+ cells was not affected. This result is predicted since SEA protein does not bind mouse Vβ8+ T cells. The decline of the percentage of CD4+, Vβ3+ T cells occurred as rapidly as in

5   mice injected with the recombinant SEA protein (rSEA). The deletion, however, observed over the next 2 months in mice injected with PCR₃-SEA.S DNA was longer lasting and was more pronounced than the deletion induced by injection of SEA.S protein. In addition, injection of as little as 2 μg PCR₃-

10   SEA.S DNA also induced deletion of Vβ3+ T cells. Thus, intramuscular injection of DNA encoding superantigens represents a potent and non-toxic approach to the deletion or suppression of potentially harmful (e.g., autoreactive T cells) T cells.

15   <u>Example 12</u>

     This example describes the production of immunogen and chemokine encoding recombinant molecules.

     Recombinant molecules encoding ovalbumin (OVA) were produced by ligating cDNA encoding OVA into the eukaryotic

20   expression vector PCR₃ and is referred to herein as PCR₃-OVA. cDNA encoding murine RANTES, murine macrophage inflammatory protein-1 alpha (MIP-1α), and macrophage inflammatory protein-1 beta (MIP-1β) was prepared from RNA isolated from LPS-stimulated normal murine bone marrow

25   macrophages using methods standard in the art. The cDNA were ligated into the expression vector PCR₃, and are referred to herein as PCR₃-RANTES, PCR₃-MIP-1α and PCR₃-MIP-

1β. All plasmid DNA were purified by cesium chloride gradient centrifugation and resuspended at 1.0 mg/ml in sterile PBS.

Example 13

5   This example demonstrates that the co-administration of adjuvant DNA and immunogen DNA stimulates antibody production against the immunogen protein.

Separate groups of 4 CB6 F1 mice per group were injected twice with the following mixtures of DNA: (1)

10   about 100 μg PCR$_3$-OVA + about 100 μg PCR$_3$-MIP-1β; (2) about 100 μg PCR$_3$-OVA + about 50 μg PCR$_3$-SEB (described in Example 1) + PCR$_3$-GM-CSF (described in Example 1); (3) about 100 μg PCR$_3$-OVA + about 100 μg PCR$_3$-RANTES; (4) about 100 μg PCR$_3$-OVA + about 100 μg PCR$_3$-SEB; (5) about 100 μg PCR$_3$-OVA +

15   about 100 μg PCR$_3$-GM-CSF; or (6) about 100 μg PCR$_3$-OVA alone. Control samples were also prepared which included 6 non-injected, syngeneic mice. The DNA was diluted to a final concentration of 0.5 mg/ml in sterile phosphate buffered saline (PBS) prior to injection. The mice were

20   injected intramuscularly, bilaterally in their quadriceps muscles (about 100 μg of DNA per quadricep).

About 20 days after the immunization of step B, serum was collected from each mouse and assayed for antibodies that specifically bind to OVA protein using an OVA-specific

25   enzyme linked immunoassay (ELISA) assay using methods standard in the art. Briefly, OVA protein was bound to an ELISA plate. The plates were washed and then incubated in

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the presence of serum. Again the plates were washed and
then incubated in the presence of HRP-conjugated anti-mouse
IgG antibody. The amount of antibody bound to the OVA was
detected on an ELISA reader and are expressed in absorbance
5   units.

The results of the ELISA are shown in Fig. 9 and
indicate that co-injection of DNA encoding OVA, with either
DNA encoding RANTES or MIP-1β, or SEB and GM-CSF, increases
the antibody response to OVA over that observed with OVA
10   alone, OVA plus GM-CSF, OVA plus SEB alone or control
samples. Thus, the expression of RANTES, MIP-1β, or SEB
and GM-CSF increase the antibody response to OVA when
administered as a DNA vaccine.

Example 14

15   This example demonstrates that the co-administration
of DNA adjuvant and immunogen DNA results in the production
of interferon gamma.

Separate groups of 4 CB6 F1 mice per group were
injected twice, intramuscularly (on day 1 and day 21), with
20   the following mixtures of DNA: (1) about 100 μg PCR$_3$-OVA +
about 100 μg PCR$_3$-MIP-1β; (2) about 100 μg PCR$_3$-OVA + about
50 μg PCR$_3$-SEB + PCR$_3$-GM-CSF; (3) about 100 μg PCR$_3$-OVA +
about 100 μg PCR$_3$-RANTES; (4) about 100 μg PCR$_3$-OVA + about
100 μg PCR$_3$-SEB; (5) about 100 μg PCR$_3$-OVA + about 100 μg
25   PCR$_3$-GM-CSF; or (6) about 100 μg PCR$_3$-OVA alone. Control
samples were also prepared as above.

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The mice were sacrificed on day 27. Spleen cells were harvested from each mouse and re-stimulated *in vitro* with irradiated OVA-transfected cells (EG7-OVA) in quadruplicate wells. On day 4 of the re-stimulation with

5 irradiated EG7-OVA cells, supernatants were harvested from the cultures and assayed for interferon gamma activity using an interferon gamma-specific ELISA assay. Results were expressed as units/ml of interferon activity, as determined by comparison with a standard curve generated

10 with recombinant murine interferon-gamma.

The results are shown in Fig. 10 and indicate that RANTES or GM-CSF were effective compounds for inducing interferon-gamma production. Although less, SEB and MIP-1β also induced interferon-gamma production. Additional

15 experiments indicated that none of the adjuvants evaluated in this experiment induced significant quantities of IL-4 release. Together, these data indicate that the immune response induced by an adjuvant of the present invention is primarily a Th1 response, which induces primarily cell-

20 mediated immunity, including macrophage activation, enhanced T cell CTL activity, and increased MHC expression.

Example 15

This example demonstrates that the co-administration of adjuvant DNA and immunogen DNA induce T cell

25 proliferative responses to the immunogen.

Separate group of 4 CB6 F1 mice per group were immunized using the protocol described in Example 14. The

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animals were sacrificed on day 27 and harvested spleen
cells re-stimulated using the method described in Example
14. After about 4 days of re-stimulation, 100 μl aliquots
of the cells were harvested from each well and pulsed for
5   18 hours with ^{3}H-thymidine. Thymidine incorporation was
then quantitated (cpm) as a measure of the proliferative
response to OVA expressed by the transfected EG7-OVA cell
line.

The results are shown in Fig. 11 and indicate that
10   MIP-1β, RANTES, SEB + GM-CSF, and SEB alone, when co-
administered together with OVA DNA, induce a substantial
increase in the proliferative response to OVA. Thus, these
data provide evidence that DNA encoding chemokines and SAgs
are useful for enhancing cell-mediated immune responses and
15   therefore are useful as DNA vaccine adjuvants.

Example 16

This example demonstrates that the co-administration
of adjuvant DNA increases CTL responses to the immunogen
ovalbumin.

20   Mice were immunized using the protocol described in
Example 14. Spleen cells were harvested from the immunized
mice 7 days after the last vaccination. The cells were
then re-stimulated in vitro for 6 days with irradiated EG7-
OVA cells. T cells were then harvested from the re-
25   stimulated population and added in decreasing numbers to
^{51}Cr-labeled EG7-OVA or EL-4 target cells in a standard 4
hour chromium release assay for CTL activity. The percent

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cell lysis was determined Chromium release was then quantitated (cpm) as a measure of the percent specific cell lysis of labeled target cells. The higher the % specific lysis, the more CTL activity exhibited by the T cells.

5   The results are shown in Fig. 12 and indicate that all of the adjuvant DNAs evaluated induced increased CTL activity compared to OVA alone. The use of RANTES, GM-CSF and SEB alone, each were effective in inducing CTL activity. These data indicate that co-administration of

10   chemokine DNA can enhance CTL-mediated immunity to an intracellular immunogen, as typified by OVA expressed in a transfected cell line, indicating that this approach is useful for vaccines against intracellular pathogens.

          Taken together, the results of Examples 12-16 indicate

15   that all DNA adjuvants tested (GM-CSF, SEB, SEB+GM-CSF, RANTES and MIP-1β) improved cell mediated immunity against the immunogen ovalbumin. In particular, the use of either SEB or GM-CSF alone, as well as the combination of SEB + GM-CSF were effective at inducing cell mediated immunity.

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SEQUENCE LISTING

The following Sequence Listing is submitted pursuant

to 37 CFR §1.821. A copy in computer readable form is also

submitted herewith.

5   Applicants assert pursuant to 37 CFR §1.821(f) that

the content of the paper and computer readable copies of

SEQ ID NO:1 through SEQ ID NO:13 submitted herewith are the

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10   (i) APPLICANT: Dow, Steve W.
                          Elmslie, Robyn E.
                          Potter, Terence A.

          (ii) TITLE OF INVENTION: GENE THERAPY FOR EFFECTOR CELL
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15   (iii) NUMBER OF SEQUENCES: 13

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    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 773 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 1..765

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATG ACC ATG ATT ACG AAT TTA ATA CGA CTC ACT ATA GGG AAT TCC ATG      48
Met Thr Met Ile Thr Asn Leu Ile Arg Leu Thr Ile Gly Asn Ser Met
 1               5                  10                  15

GAG AGT CAA CCA GAT CCT AAA CCA GAT GAG TTG CAC AAA TCG AGT AAA      96
Glu Ser Gln Pro Asp Pro Lys Pro Asp Glu Leu His Lys Ser Ser Lys
            20                  25                  30

TTC ACT GGT TTG ATG GAA AAT ATG AAA GTT TTG TAT GAT GAT AAT CAT     144
Phe Thr Gly Leu Met Glu Asn Met Lys Val Leu Tyr Asp Asp Asn His
        35                  40                  45

GTA TCA GCA ATA AAC GTT AAA TCT ATA GAT CAA TTT CTA TAC TTT GAC     192
Val Ser Ala Ile Asn Val Lys Ser Ile Asp Gln Phe Leu Tyr Phe Asp
    50                  55                  60

TTA ATA TAT TCT ATT AAG GAC ACT AAG TTA GGG AAT TAT GAT AAT GTT     240
Leu Ile Tyr Ser Ile Lys Asp Thr Lys Leu Gly Asn Tyr Asp Asn Val
65                  70                  75                  80

CGA GTC GAA TTT AAA AAC AAA GAT TTA GCT GAT AAA TAC AAA GAT AAA     288
Arg Val Glu Phe Lys Asn Lys Asp Leu Ala Asp Lys Tyr Lys Asp Lys
                85                  90                  95

TAC GTA GAT GTG TTT GGA GCT AAT TAT TAT TAT CAA TGT TAT TTT TCT     336
Tyr Val Asp Val Phe Gly Ala Asn Tyr Tyr Tyr Gln Cys Tyr Phe Ser
            100                 105                 110

AAA AAA ACG AAT GAT ATT AAT TCG CAT CAA ACT GAC AAA CGA AAA ACT     384
Lys Lys Thr Asn Asp Ile Asn Ser His Gln Thr Asp Lys Arg Lys Thr
        115                 120                 125

TGT ATG TAT GGT GGT GTA ACT GAG CAT AAT GGA AAC CAA TTA GAT AAA     432
Cys Met Tyr Gly Gly Val Thr Glu His Asn Gly Asn Gln Leu Asp Lys
    130                 135                 140

TAT AGA AGT ATT ACT GTT CGG GTA TTT GAA GAT GGT AAA AAT TTA TTA     480
Tyr Arg Ser Ile Thr Val Arg Val Phe Glu Asp Gly Lys Asn Leu Leu
145                 150                 155                 160

TCT TTT GAC GTA CAA ACT AAT AAG AAA AAG GTG ACT GCT CAA GAA TTA     528
Ser Phe Asp Val Gln Thr Asn Lys Lys Lys Val Thr Ala Gln Glu Leu
                165                 170                 175

GAT TAC CTA ACT CGT CAC TAT TTG GTG AAA AAT AAA AAA CTC TAT GAA     576
Asp Tyr Leu Thr Arg His Tyr Leu Val Lys Asn Lys Lys Leu Tyr Glu
            180                 185                 190
```

```
TTT AAC AAC TCG CCT TAT GAA ACG GGA TAT ATT AAA TTT ATA GAA AAT    624
Phe Asn Asn Ser Pro Tyr Glu Thr Gly Tyr Ile Lys Phe Ile Glu Asn
        195             200             205

GAG AAT AGC TTT TGG TAT GAC ATG ATG CCT GCA CCA GGA GAT AAA TTT    672
Glu Asn Ser Phe Trp Tyr Asp Met Met Pro Ala Pro Gly Asp Lys Phe
        210             215             220

GAC CAA TCT AAA TAT TTA ATG ATG TAC AAT GAC AAT AAA ATG GTT GAT    720
Asp Gln Ser Lys Tyr Leu Met Met Tyr Asn Asp Asn Lys Met Val Asp
225             230             235             240

TCT AAA GAT GTG AAG ATT GAA GTT TAT CTT ACG ACA AAG AAA AAG        765
Ser Lys Asp Val Lys Ile Glu Val Tyr Leu Thr Thr Lys Lys Lys
            245             250             255

TGAAGCTT                                                           773
```

(2) INFORMATION FOR SEQ ID NO:2:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 255 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: protein

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Thr Met Ile Thr Asn Leu Ile Arg Leu Thr Ile Gly Asn Ser Met
 1           5               10              15

Glu Ser Gln Pro Asp Pro Lys Pro Asp Glu Leu His Lys Ser Ser Lys
            20              25              30

Phe Thr Gly Leu Met Glu Asn Met Lys Val Leu Tyr Asp Asp Asn His
            35              40              45

Val Ser Ala Ile Asn Val Lys Ser Ile Asp Gln Phe Leu Tyr Phe Asp
        50              55              60

Leu Ile Tyr Ser Ile Lys Asp Thr Lys Leu Gly Asn Tyr Asp Asn Val
 65              70              75              80

Arg Val Glu Phe Lys Asn Lys Asp Leu Ala Asp Lys Tyr Lys Asp Lys
            85              90              95

Tyr Val Asp Val Phe Gly Ala Asn Tyr Tyr Tyr Gln Cys Tyr Phe Ser
            100             105             110

Lys Lys Thr Asn Asp Ile Asn Ser His Gln Thr Asp Lys Arg Lys Thr
            115             120             125

Cys Met Tyr Gly Gly Val Thr Glu His Asn Gly Asn Gln Leu Asp Lys
        130             135             140

Tyr Arg Ser Ile Thr Val Arg Val Phe Glu Asp Gly Lys Asn Leu Leu
145             150             155             160

Ser Phe Asp Val Gln Thr Asn Lys Lys Lys Val Thr Ala Gln Glu Leu
            165             170             175

Asp Tyr Leu Thr Arg His Tyr Leu Val Lys Asn Lys Lys Leu Tyr Glu
            180             185             190
```

```
         Phe Asn Asn Ser Pro Tyr Glu Thr Gly Tyr Ile Lys Phe Ile Glu Asn
                 195             200             205

         Glu Asn Ser Phe Trp Tyr Asp Met Met Pro Ala Pro Gly Asp Lys Phe
                 210             215             220

      5  Asp Gln Ser Lys Tyr Leu Met Met Tyr Asn Asp Asn Lys Met Val Asp
         225             230             235             240

         Ser Lys Asp Val Lys Ile Glu Val Tyr Leu Thr Thr Lys Lys Lys
                 245             250             255
```

      (2) INFORMATION FOR SEQ ID NO:3:

10   (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 751 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear

15   (ii) MOLECULE TYPE: protein

           (ix) FEATURE:
                (A) NAME/KEY: CDS
                (B) LOCATION: 46..744

           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
20   ATGACCATGA TTACGAATTT AATACGACTC ACTATAGGGA ATTCC ATG GAG AAA       54
                                                     Met Glu Lys
                                                      1

     AGC GAA GAA ATA AAT GAG AAA GAT CTG CGC AAG AAG TCC GAA TTG CAG    102
     Ser Glu Glu Ile Asn Glu Lys Asp Leu Arg Lys Lys Ser Glu Leu Gln
25       5               10              15

     GGA ACA GCC CTA GGC AAT CTT AAA CAA ATC TAT TAT TAC AAT GAA AAA    150
     Gly Thr Ala Leu Gly Asn Leu Lys Gln Ile Tyr Tyr Tyr Asn Glu Lys
         20              25              30              35

     GCG AAG ACT GAG AAT AAA GAG AGT CAC GAT CAA TTT CTG CAG CAT ACT    198
30   Ala Lys Thr Glu Asn Lys Glu Ser His Asp Gln Phe Leu Gln His Thr
                 40              45              50

     ATA TTG TTT AAA GGC TTT TTT ACT GAT CAT TCG TGG TAT AAC GAT TTA    246
     Ile Leu Phe Lys Gly Phe Phe Thr Asp His Ser Trp Tyr Asn Asp Leu
                 55              60              65

35   CTA GTA GAT TTT GAT TCG AAG GAC ATC GTT GAT AAA TAT AAA GGG AAG    294
     Leu Val Asp Phe Asp Ser Lys Asp Ile Val Asp Lys Tyr Lys Gly Lys
                 70              75              80

     AAG GTC GAC TTG TAT GGT GCT TAT TAT GGG TAC CAA TGT GCT GGT GGT    342
     Lys Val Asp Leu Tyr Gly Ala Tyr Tyr Gly Tyr Gln Cys Ala Gly Gly
40       85              90              95

     ACA CCA AAC AAA ACA GCA TGC ATG TAT GGT GGG GTA ACC TTA CAT GAC    390
     Thr Pro Asn Lys Thr Ala Cys Met Tyr Gly Gly Val Thr Leu His Asp
     100             105             110             115

     AAT AAT CGA TTG ACC GAA GAG AAA AAG GTC CCG ATC AAT TTA TGG CTA    438
45   Asn Asn Arg Leu Thr Glu Glu Lys Lys Val Pro Ile Asn Leu Trp Leu
                 120             125             130
```

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```
GAC GGT AAA CAA AAT ACA GTA CCT CTA GAA ACG GTT AAA ACG AAT AAG     486
Asp Gly Lys Gln Asn Thr Val Pro Leu Glu Thr Val Lys Thr Asn Lys
        135             140                 145

AAA AAT GTA ACT GTC CAA GAG CTG GAT CTT CAA GCG CGC CGA TAC CTA     534
Lys Asn Val Thr Val Gln Glu Leu Asp Leu Gln Ala Arg Arg Tyr Leu
        150             155             160

CAG GAA AAA TAT AAT TTG TAC AAC TCT GAC GTC TTT GAT GGG AAG GTT     582
Gln Glu Lys Tyr Asn Leu Tyr Asn Ser Asp Val Phe Asp Gly Lys Val
        165             170             175

CAG AGA GGC CTA ATC GTG TTT CAT ACT TCT ACA GAA CCT TCG GTT AAC     630
Gln Arg Gly Leu Ile Val Phe His Thr Ser Thr Glu Pro Ser Val Asn
180             185             190             195

TAC GAT TTA TTT GGA GCT CAA GGA CAG TAT TCA AAT ACA CTC TTA AGA     678
Tyr Asp Leu Phe Gly Ala Gln Gly Gln Tyr Ser Asn Thr Leu Leu Arg
                200             205             210

ATA TAT CGC GAC AAC AAG ACG ATT AAC TCT GAA AAC ATG CAC ATT GAT     726
Ile Tyr Arg Asp Asn Lys Thr Ile Asn Ser Glu Asn Met His Ile Asp
            215             220             225

ATC TAT TTA TAT ACA AGT TAAGCTT                                     751
Ile Tyr Leu Tyr Thr Ser
            230
```

(2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 233 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Glu Lys Ser Glu Glu Ile Asn Glu Lys Asp Leu Arg Lys Lys Ser
 1            5                10           Leu         15

Glu Leu Gln Gly Thr Ala Leu Gly Asn Leu Lys Gln Ile Tyr Tyr Tyr
          20              25              30

Asn Glu Lys Ala Lys Thr Glu Asn Lys Glu Ser His Asp Gln Phe Leu
        35              40              45

Gln His Thr Ile Leu Phe Lys Gly Phe Phe Thr Asp His Ser Trp Tyr
        50              55              60

Asn Asp Leu Leu Val Asp Phe Asp Ser Lys Asp Ile Val Asp Lys Tyr
        65              70              75              80

Lys Gly Lys Lys Val Asp Leu Tyr Gly Ala Tyr Tyr Gly Tyr Gln Cys
                85              90              95

Ala Gly Gly Thr Pro Asn Lys Thr Ala Cys Met Tyr Gly Gly Val Thr
                100             105             110

Leu His Asp Asn Asn Arg Leu Thr Glu Glu Lys Lys Val Pro Ile Asn
            115             120             125

Leu Trp Leu Asp Gly Lys Gln Asn Thr Val Pro Leu Glu Thr Val Lys
            130             135             140
```

```
Thr Asn Lys Lys Asn Val Thr Val Gln Glu Leu Asp Leu Gln Ala Arg
145             150             155             160
```

```
Arg Tyr Leu Gln Glu Lys Tyr Asn Leu Tyr Asn Ser Asp Val Phe Asp
            165             170             175
```

5
```
Gly Lys Val Gln Arg Gly Leu Ile Val Phe His Thr Ser Thr Glu Pro
            180             185             190
```

```
Ser Val Asn Tyr Asp Leu Phe Gly Ala Gln Gly Gln Tyr Ser Asn Thr
            195             200             205
```

10
```
Leu Leu Arg Ile Tyr Arg Asp Asn Lys Thr Ile Asn Ser Glu Asn Met
    210             215             220
```

```
His Ile Asp Ile Tyr Leu Tyr Thr Ser
225             230
```

(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:

15
        (A) LENGTH: 582 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

20
    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 1..582

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
ATG ACA AAC GAT AAT ATA AAG GAT TTG CTA GAC TGG TAT AGT AGT GGG    48
```
25
```
Met Thr Asn Asp Asn Ile Lys Asp Leu Leu Asp Trp Tyr Ser Ser Gly
  1           5               10              15
```

```
TCT GAC ACT TTT ACA AAT AGT GAA GTT TTA GAT AAT TCC TTA GGA TCT    96
Ser Asp Thr Phe Thr Asn Ser Glu Val Leu Asp Asn Ser Leu Gly Ser
            20              25              30
```

30
```
ATG CGT ATA AAA AAC ACA GAT GGC AGC ATC AGC CTT ATA ATT TTT CCG   144
Met Arg Ile Lys Asn Thr Asp Gly Ser Ile Ser Leu Ile Ile Phe Pro
            35              40              45
```

```
AGT CCT TAT TAT AGC CCT GCT TTT ACA AAA GGG GAA AAA GTT GAC TTA   192
Ser Pro Tyr Tyr Ser Pro Ala Phe Thr Lys Gly Glu Lys Val Asp Leu
35          50              55              60
```

```
AAC ACA AAA AGA ACT AAA AAA AGC CAA CAT ACT AGC GAA GGA ACT TAT   240
Asn Thr Lys Arg Thr Lys Lys Ser Gln His Thr Ser Glu Gly Thr Tyr
65              70              75              80
```

```
ATC CAT TTC CAA ATA AGT GGC GTT ACA AAT ACT GAA AAA TTA CCT ACT   288
40
Ile His Phe Gln Ile Ser Gly Val Thr Asn Thr Glu Lys Leu Pro Thr
                85              90              95
```

```
CCA ATA GAA CTA CCT TTA AAA GTT AAG GTT CAT GGT AAA GAT AGC CCC   336
Pro Ile Glu Leu Pro Leu Lys Val Lys Val His Gly Lys Asp Ser Pro
            100             105             110
```

45
```
TTA AAG TAT TGG CCA AAG TTC GAT AAA AAA CAA TTA GCT ATA TCA ACT   384
Leu Lys Tyr Trp Pro Lys Phe Asp Lys Lys Gln Leu Ala Ile Ser Thr
            115             120             125
```

```
     TTA GAC TTT GAA ATT CGT CAT CAG CTA ACT CAA ATA CAT GGA TTA TAT    432
     Leu Asp Phe Glu Ile Arg His Gln Leu Thr Gln Ile His Gly Leu Tyr
         130                 135                 140

 5   CGT TCA AGC GAT AAA ACG GGT GGT TAT TGG AAA ATA ACA ATG AAT GAC    480
     Arg Ser Ser Asp Lys Thr Gly Gly Tyr Trp Lys Ile Thr Met Asn Asp
     145                 150                 155                 160

     GGA TCC ACA TAT CAA AGT GAT TTA TCT AAA AAG TTT GAA TAC AAT ACT    528
     Gly Ser Thr Tyr Gln Ser Asp Leu Ser Lys Lys Phe Glu Tyr Asn Thr
                     165                 170                 175

10   GAA AAA CCA CCT ATA AAT ATT GAT GAA ATA AAA ACT ATA GAA GCA GAA    576
     Glu Lys Pro Pro Ile Asn Ile Asp Glu Ile Lys Thr Ile Glu Ala Glu
                 180                 185                 190

     ATT AAT                                                            582
     Ile Asn
15
     (2) INFORMATION FOR SEQ ID NO:6:

         (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 194 amino acids
             (B) TYPE: amino acid
20           (D) TOPOLOGY: linear

         (ii) MOLECULE TYPE: protein

         (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

     Met Thr Asn Asp Asn Ile Lys Asp Leu Leu Asp Trp Tyr Ser Ser Gly
      1               5                  10                  15

25   Ser Asp Thr Phe Thr Asn Ser Glu Val Leu Asp Asn Ser Leu Gly Ser
                     20                  25                  30

     Met Arg Ile Lys Asn Thr Asp Gly Ser Ile Ser Leu Ile Ile Phe Pro
             35                  40                  45

     Ser Pro Tyr Tyr Ser Pro Ala Phe Thr Lys Gly Glu Lys Val Asp Leu
30       50                  55                  60

     Asn Thr Lys Arg Thr Lys Lys Ser Gln His Thr Ser Glu Gly Thr Tyr
     65                  70                  75                  80

     Ile His Phe Gln Ile Ser Gly Val Thr Asn Thr Glu Lys Leu Pro Thr
                         85                  90                  95

35   Pro Ile Glu Leu Pro Leu Lys Val Lys Val His Gly Lys Asp Ser Pro
                     100                 105                 110

     Leu Lys Tyr Trp Pro Lys Phe Asp Lys Lys Gln Leu Ala Ile Ser Thr
             115                 120                 125

     Leu Asp Phe Glu Ile Arg His Gln Leu Thr Gln Ile His Gly Leu Tyr
40       130                 135                 140

     Arg Ser Ser Asp Lys Thr Gly Gly Tyr Trp Lys Ile Thr Met Asn Asp
     145                 150                 155                 160

     Gly Ser Thr Tyr Gln Ser Asp Leu Ser Lys Lys Phe Glu Tyr Asn Thr
                     165                 170                 175

45   Glu Lys Pro Pro Ile Asn Ile Asp Glu Ile Lys Thr Ile Glu Ala Glu
                 180                 185                 190

     Ile Asn
```

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(2) INFORMATION FOR SEQ ID NO:7:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 base pairs
5   (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
10   (A) NAME/KEY: misc_feature
            (B) LOCATION: 1..25
            (D) OTHER INFORMATION: /label= primer

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGGAATTCCA TGGAGAGTCA ACCAG   25

15   (2) INFORMATION FOR SEQ ID NO:8:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 23 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
20   (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 1..23
25   (D) OTHER INFORMATION: /label= primer

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCGGATCCTC ACTTTTTCTT TGT   23

    (2) INFORMATION FOR SEQ ID NO:9:

        (i) SEQUENCE CHARACTERISTICS:
30   (A) LENGTH: 22 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

35   (ix) FEATURE:
            (A) NAME/KEY: misc_feature
            (B) LOCATION: 1..22
            (D) OTHER INFORMATION: /label= primer

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

40 GGGAATTCCA TGGAGAAAAG CG   22

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(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 25 base pairs
        (B) TYPE: nucleic acid
5   (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
        (A) NAME/KEY: misc_feature
10   (B) LOCATION: 1..25
        (D) OTHER INFORMATION: /label= primer

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCAAGCTTAA CTTGTATATA AATAG   25

(2) INFORMATION FOR SEQ ID NO:11:

15   (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 51 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

20   (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
        (A) NAME/KEY: misc_feature
        (B) LOCATION: 1..51

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

25 CGGGGTACCC CGAAGGAGGA AAAAAAAATG TCTACAAACG ATAATATAAA G   51

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 42 base pairs
        (B) TYPE: nucleic acid
30   (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (ix) FEATURE:
        (A) NAME/KEY: misc_feature
35   (B) LOCATION: 1..42
        (D) OTHER INFORMATION: /label= primer

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TGCTCTAGAG CATTAATTAA TTTCTGCTTC TATAGTTTTT AT   42

(2) INFORMATION FOR SEQ ID NO:13:

      (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 279 base pairs
        (B) TYPE: nucleic acid
5   (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA (genomic)

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ACCATGAAGA TCTCTGCAGC TGCCCTCACC ATCATCCTCA CTGCAGCCGC CCTCTGGGCG   60

10 CCCGCGCCTG CCTCACCATA TGGCTCGGAC ACCACTCCCT GCTGCTTTGC CTACCTCTCC 120

CTCGCGCTGC CTCGTGCCCA CGTCAAGGAG TATTTCTACA CCAGCAGCAA GTGCTCCAAT 180

CTTGCAGTCG TGTTTGTCAC TCGAAGGAAC CGCCAAGTGT GTGCCAACCC AGAGAAGAAG 240

TGGGTTCAAG AATACATCAA CTATTTGGAG ATGAGCTAG   279

      While various embodiments of the present invention

15 have been described in detail, it is apparent that

modifications and adaptations of those embodiments will

occur to those skilled in the art. It is to be expressly

understood, however, that such modifications and

adaptations are within the scope of the present invention,

20 as set forth in the following claims:

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What is claimed:

1.   A therapeutic composition comprising: (a) an isolated nucleic acid molecule encoding a superantigen; and (b) an isolated nucleic acid molecule selected from the
5   group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine and mixtures thereof, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

10   2.   The therapeutic composition of Claim 1, wherein said superantigen is selected from the group consisting of staphylococcal enterotoxins, retroviral antigens, streptococcal antigens, mycoplasma antigens, mycobacteria antigens, viral antigens and protozoan antigens.

15   3.   The therapeutic composition of Claim 1, wherein said superantigen comprises staphylococcal enterotoxins.

4.   The therapeutic composition of Claim 1, wherein said superantigen is selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

20   5.   The therapeutic composition of Claim 1, wherein said superantigen is derived from a virus selected from the group consisting of mouse mammary tumor virus, rabies virus and herpes virus.

6.   The therapeutic composition of Claim 1, wherein
25   said cytokine is selected from the group consisting of hematopoietic growth factors, interleukins, interferons,

immunoglobulin superfamily molecules, tumor necrosis factor family molecules and chemokines.

7.   The therapeutic composition of Claim 1, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, and interleukin-15.

8.   The therapeutic composition of Claim 1, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor and tumor necrosis factor α.

9.   The therapeutic composition of Claim 1, wherein said cytokine comprises granulocyte macrophage colony stimulating factor.

10.   The therapeutic composition of Claim 1, wherein said chemokine is selected from the group consisting of C5a, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB$_4$R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

11.   The therapeutic composition of Claim 1, wherein said chemokine is selected from the group consisting of IL-8, MIP1α, MIP1β, MCP-1, MCP-3, RANTES and NAP-2.

12.   The therapeutic composition of Claim 1, wherein said chemokine is selected from the group consisting of IL-8, Rantes, MIP1α and MIP1β.

13.   The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences capable of being expressed in a mammalian cell.

5   14.   The therapeutic composition of Claim 1, wherein said transcription control sequences are selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR sequences, SV-40 control sequences and β-actin control sequences.

10   15.   The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecule encoding a superantigen comprises a recombinant molecule selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST.

15   16.   The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecule encoding a cytokine comprises PCR_3-GM_3.

         17.   The therapeutic composition of Claim 1, wherein said isolated nucleic acid molecule encoding a chemokine is

20   selected from the group consisting of PCR_3-RANTES, PCR_3-MIP1α and PCR_3-MIP1β.

         18.   The therapeutic composition of Claim 1, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier selected from the group

25   consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-

containing substrate, an oil, an ester, a glycol, a virus and a metal particle.

19. The therapeutic composition of Claim 18, wherein said pharmaceutically acceptable carrier is selected from the group consisting of liposomes and an aqueous physiologically balanced solution.

20. The therapeutic composition of Claim 1, wherein said therapeutic composition further comprises an immunogen selected from the group consisting of a pathogen, an allergen, tumor antigens and self-antigens.

21. The therapeutic composition of Claim 1, wherein said therapeutic composition further comprises an immunogen comprising a peptide derived from *Mycobacterium tuberculosis*.

22. A recombinant molecule comprising: (a) a first isolated nucleic acid molecule encoding a superantigen; and (b) a second isolated nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

23. The molecule of Claim 22, wherein said recombinant molecule contains a transcription control sequence selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR control

sequences, SV-40 control sequences and β-actin control sequences.

24. The molecule of Claim 22, wherein said recombinant molecule is dicistronic and comprises an IRES.

25. The molecule of Claim 22, wherein said first nucleic acid molecule and said second nucleic acid molecule are separated by an IRES.

26. The molecule of Claim 22, wherein said first nucleic acid molecule encodes a superantigen selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

27. The molecule of Claim 22, wherein said second nucleic acid molecule encodes a protein selected from the group consisting of macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, interleukin-15, C5a, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB_4R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

28. A recombinant molecule comprising: (a) a first isolated nucleic acid molecule encoding a first superantigen; and (b) a second isolated nucleic acid molecule encoding a second superantigen, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

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29. The molecule of Claim 28, wherein said recombinant molecule contains a transcription control sequence selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR control

5   sequences, SV-40 control sequences and β-actin control sequences.

30. The molecule of Claim 28, wherein said recombinant molecule is dicistronic and comprises an IRES.

31. The molecule of Claim 28, wherein said first

10   nucleic acid molecule and said second nucleic acid molecule are separated by an IRES.

32. The molecule of Claim 28, wherein said first nucleic acid molecule encodes a superantigen selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE

15   and TSST.

33. A therapeutic composition comprising a delivery vehicle carrying: (a) a first isolated nucleic acid molecule encoding a superantigen; and (b) a second isolated nucleic acid molecule selected from the group consisting of

20   a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine and mixtures thereof, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

34. The therapeutic composition of Claim 33, wherein

25   said delivery vehicle comprises a liposome.

35. The therapeutic composition of Claim 33, wherein said first nucleic acid molecule encodes a superantigen

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selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

36.   The therapeutic composition of Claim 33, wherein said second nucleic acid molecule encodes a protein selected from the group consisting of macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, interleukin-15, C5a, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB_4R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

37.   The therapeutic composition of Claim 33, wherein said delivery vehicle further carries a nucleic acid molecule encoding an immunogen.

38.   The therapeutic composition of Claim 33, wherein said delivery vehicle further carries a peptide derived from *Mycobacterium tuberculosis*.

39.   A therapeutic composition comprising a delivery vehicle carrying an isolated nucleic acid molecule encoding a superantigen, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

40.   The therapeutic composition of Claim 39, wherein said delivery vehicle comprises a liposome.

41.   The therapeutic composition of Claim 39, wherein said nucleic acid molecule encodes a superantigen selected

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from the group consisting of SEA, SEB, SEC$_1$, SEC$_2$, SEC$_3$, SED, SEE and TSST.

42. The therapeutic composition of Claim 39, wherein said delivery vehicle further carries a nucleic acid molecule encoding a protein selected from the group consisting of a cytokine, a chemokine and an immunogen.

43. The therapeutic composition of Claim 39, wherein said delivery vehicle further carries a nucleic acid molecule encoding a protein selected from the group consisting of macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-2, interleukin-4, interleukin-6, interleukin-12, interleukin-15, C5a, IL-8, MIP1α, MIP1β, MCP-1, MCP-3, PAFR, FMLPR, LTB$_4$R, GRP, RANTES, eotaxin, lymphotactin, IP10, I-309, ENA78, GCP-2, NAP-2 and MGSA/gro.

44. The therapeutic composition of Claim 39, wherein said delivery vehicle further carries a peptide derived from *Mycobacterium tuberculosis*.

45. An adjuvant composition, comprising an immunogen and an isolated nucleic acid molecule encoding a superantigen, wherein said isolated nucleic acid molecule is operatively linked to one or more transcription control sequences.

46. The adjuvant composition of Claim 45, wherein said adjuvant composition further comprises a nucleic acid

molecule encoding a protein selected from the group consisting of a cytokine, a chemokine and mixtures thereof.

47. The adjuvant composition of Claim 45, wherein said immunogen comprises a compound selected from the group consisting of a nucleic acid molecule and a peptide.

48. The adjuvant composition of Claim 45, wherein said adjuvant composition comprises up to about 50% of a nucleic acid molecule encoding an immunogen and up to about 50% of a nucleic acid molecule encoding a superantigen.

49. The adjuvant composition of Claim 45, wherein said adjuvant composition comprises up to about 66% of a nucleic acid molecule encoding an immunogen and up to about 33% of a nucleic acid molecule encoding a superantigen.

50. The adjuvant composition of Claim 45, wherein said adjuvant composition comprises up to about 50% of a nucleic acid molecule encoding an immunogen, up to about 25% of a nucleic acid molecule encoding a superantigen and up to about 25% of a nucleic acid molecule selected from the group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine and mixtures thereof.

51. The adjuvant composition of Claim 45, wherein said nucleic acid molecules comprise naked DNA.

52. The adjuvant composition of Claim 45, wherein said composition further comprises a peptide derived from *Mycobacterium tuberculosis*.

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53. A method for increasing effector cell immunity in an animal, said method comprising administering to an animal an effective amount of an adjuvant composition comprising an immunogen and an isolated nucleic acid

5   molecule encoding a superantigen.

54. The method of Claim 53, wherein said adjuvant composition further comprises a nucleic acid molecule encoding a protein selected from the group consisting of a cytokine, a chemokine and mixtures thereof.

10   55. The method of Claim 53, wherein said adjuvant composition further comprises a pharmaceutically acceptable carrier.

56. The method of Claim 53, wherein said step of administering comprises injecting said adjuvant composition

15   by a route selected from the group consisting of intravenous, intraperitoneally, intramuscularly, intraarterially and directly into a specific tissue site.

57. The method of Claim 53, wherein said animal is a mammal.

20   58. The method of Claim 53, wherein said animal is selected from the from the group consisting of humans, horses, dogs, cats and cattle.

59. A method to treat an animal with cancer, said method comprising administering to an animal an effective

25   amount of a therapeutic composition comprising: (a) an isolated nucleic acid molecule encoding a superantigen; and (b) an isolated nucleic acid molecule selected from the

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group consisting of a nucleic acid molecule encoding a cytokine, a nucleic acid molecule encoding a chemokine, and mixtures thereof, wherein said nucleic acid molecules are operatively linked to one or more transcription control
5   sequences.

60. The method of Claim 59, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-
10   containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and a metal particle.

61. The method of Claim 59, wherein said pharmaceutically acceptable carrier is selected from the group consisting of liposomes, micelles, cells and cellular
15   membranes.

62. The method of Claim 59, wherein said pharmaceutically acceptable carrier comprises a liposome.

63. The method of Claim 59, wherein said pharmaceutically acceptable carrier comprises a liposome
20   that includes a compound capable of specifically targeting said liposome to a tumor cell.

64. The method of Claim 63, wherein said compound is a tumor cell ligand.

65. The method of Claim 59, wherein said therapeutic
25   composition is targeted to the site of a cancer in said animal by administering said therapeutic composition locally within the area of a cancer cell.

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66. The method of Claim 59, wherein said cancer is selected from the group consisting of melanomas, squamous cell carcinoma, breast cancers, head and neck carcinomas, thyroid carcinomas, soft tissue sarcomas, bone sarcomas,

5 testicular cancers, prostatic cancers, ovarian cancers, bladder cancers, skin cancers, brain cancers, angiosarcomas, hemangiosarcomas, mast cell tumors, primary hepatic cancers, lung cancers, pancreatic cancers, gastrointestinal cancers, renal cell carcinomas,

10 hematopoietic neoplasias, leukemias and lymphomas.

67. A method to treat an animal with cancer, said method comprising introducing into a tumor cell *in vivo* an effective amount of a therapeutic composition comprising an isolated nucleic acid molecule encoding a superantigen,

15 wherein said nucleic acid molecules are operatively linked to one or more transcription control sequences.

68. The method of Claim 67, wherein said isolated nucleic acid molecule encoding a superantigen comprises a recombinant molecule selected from the group consisting of

20 PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST.

69. The method of Claim 67, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier comprising a liposome.

70. The method of Claim 67, wherein said therapeutic

25 composition further comprises a pharmaceutically acceptable carrier comprising a liposome that includes a compound

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capable of specifically targeting said liposome to a tumor cell.

71. The method of Claim 70, wherein said compound is a tumor cell ligand.

5   72. The method of Claim 67, wherein said therapeutic composition is targeted to the site of a cancer in said animal by administering said therapeutic composition locally within the area of a cancer cell.

73. The method of Claim 67, wherein said therapeutic

10   composition is administered to said animal at a site comprising a lymph node.

74. A method to treat an animal with cancer, said method comprising introducing into a non-tumor cell an effective amount of a therapeutic composition comprising an

15   isolated nucleic acid molecule encoding a superantigen, wherein said nucleic acid molecules are operatively linked to one or more transcription control sequences.

75. The method of Claim 74, wherein said step of administration is performed *in vivo*.

20   76. The method of Claim 74, wherein said isolated nucleic acid molecule encoding a superantigen comprises a recombinant molecule selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S and PCR_3-TSST.

77. The method of Claim 74, wherein said therapeutic

25   composition comprises a pharmaceutically acceptable carrier comprising a liposome.

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78. The method of Claim 74, wherein said therapeutic composition is targeted to the site of a cancer in said animal by administering said therapeutic composition locally within the area of a cancer cell.

5   79. The method of Claim 74, wherein said therapeutic composition is administered to said animal at a site comprising a lymph node.

80. A method to treat an animal with cancer, said method comprising administering to an animal an effective

10   amount of a therapeutic composition comprising: (a) an isolated nucleic acid molecule encoding a superantigen; and (b) an isolated nucleic acid molecule encoding a cytokine, wherein said nucleic acid molecules are operatively linked to one or more transcription control sequences, and wherein

15   said therapeutic composition is targeted to the site of a cancer in said animal to treat said cancer.

81. The method of Claim 80, wherein said superantigen is selected from the group consisting of staphylococcal enterotoxins, retroviral antigens, streptococcal antigens,

20   mycoplasma antigens, mycobacteria antigens, viral antigens and protozoan antigens.

82. The method of Claim 80, wherein said superantigen comprises staphylococcal enterotoxins.

83. The method of Claim 80, wherein said superantigen

25   is selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

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84. The method of Claim 80, wherein said superantigen is derived from a virus selected from the group consisting of mouse mammary tumor virus, rabies virus and herpes virus.

5   85. The method of Claim 80, wherein said cytokine is selected from the group consisting of hematopoietic growth factors, interleukins, interferons, immunoglobulin superfamily molecules, tumor necrosis factor family molecules and chemokines.

10   86. The method of Claim 80, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-6 and interleukin-12.

15   87. The method of Claim 80, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor and tumor necrosis factor α.

88. The method of Claim 80, wherein said cytokine

20   comprises granulocyte macrophage colony stimulating factor.

89. The method of Claim 80, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences capable of being expressed in a mammalian cell.

25   90. The method of Claim 80, wherein said transcription control sequences are selected from the group consisting of RSV control sequences, CMV control sequences,

retroviral LTR sequences, SV-40 control sequences and β-actin control sequences.

91. The method of Claim 80, wherein said isolated nucleic acid molecule comprises a recombinant molecule selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST and PCR_3-GM_3.

92. The method of Claim 80, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and a metal particle.

93. The method of Claim 92, wherein said pharmaceutically acceptable carrier is selected from the group consisting of liposomes, micelles, cells and cellular membranes.

94. The method of Claim 92, wherein said pharmaceutically acceptable carrier comprises a liposome.

95. The method of Claim 92, wherein said pharmaceutically acceptable carrier comprises a liposome that includes a compound capable of specifically targeting said liposome to a tumor cell.

96. The method of Claim 95, wherein said compound is a tumor cell ligand.

97. The method of Claim 80, wherein said therapeutic composition is targeted to the site of a cancer in said

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animal by administering said therapeutic composition locally within the area of a cancer cell.

98. The method of Claim 80, wherein said cancer is selected from the group consisting of melanomas, squamous cell carcinoma, breast cancers, head and neck carcinomas, thyroid carcinomas, soft tissue sarcomas, bone sarcomas, testicular cancers, prostatic cancers, ovarian cancers, bladder cancers, skin cancers, brain cancers, angiosarcomas, hemangiosarcomas, mast cell tumors, primary hepatic cancers, lung cancers, pancreatic cancers, gastrointestinal cancers, renal cell carcinomas, hematopoietic neoplasias, leukemias and lymphomas.

99. The method of Claim 80, wherein said cancer is selected from the group consisting of melanomas, lung cancers, thyroid carcinomas, breast cancers, renal cell carcinomas, squamous cell carcinomas, brain tumors and skin cancers.

100. The method of Claim 80, wherein said animal is selected from the group consisting of mammals and birds.

101. The method of Claim 80, wherein said animal is selected from the from the group consisting of humans, house pets, economic produce animals and zoo animals.

102. The method of Claim 80, wherein said animal is selected from the from the group consisting of humans, dogs, cats, sheep, cattle, horses and pigs.

103. A therapeutic composition comprising an isolated nucleic acid molecule encoding a superantigen and an

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isolated nucleic acid molecule encoding a cytokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

104. The composition of Claim 103, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier.

105. The composition of Claim 104, wherein said pharmaceutically acceptable carrier is selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and metal particles.

106. The composition of Claim 104, wherein said pharmaceutically acceptable carrier comprises a delivery vehicle capable of delivering said nucleic acid molecules to a targeted site in an animal.

107. The composition of Claim 106, wherein said delivery vehicle is selected from the group consisting of liposomes, micelles, cells and cellular membranes.

108. The composition of Claim 106, wherein said delivery vehicle comprises a liposome.

109. The composition of Claim 106, wherein said delivery vehicle comprises a tumor cell ligand.

110. The composition of Claim 103, wherein said superantigen is selected from the group consisting of staphylococcal enterotoxins, retroviral antigens,

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streptococcal antigens, mycoplasma antigens, mycobacteria antigens, virus antigens and protozoan antigens.

111. The composition of Claim 103, wherein said superantigen comprises staphylococcal enterotoxins.

112. The composition of Claim 103, wherein said superantigen is selected from the group consisting of SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST.

113. The composition of Claim 103, wherein said superantigen is derived from a virus selected from the group consisting of mouse mammary tumor virus, rabies virus and herpes virus.

114. The composition of Claim 103, wherein said cytokine is selected from the group consisting of hematopoietic growth factors, interleukins, interferons, immunoglobulin superfamily molecules, tumor necrosis factor family molecules and chemokines.

115. The composition of Claim 103, wherein said cytokine is selected from the group consisting of granulocyte macrophage colony stimulating factor, macrophage colony stimulating factor, tumor necrosis factor α, interleukin-1, interleukin-6 and interleukin-12.

116. The composition of Claim 103, wherein said cytokine is granulocyte macrophage colony stimulating factor and tumor necrosis factor α.

117. The composition of Claim 103, wherein said isolated nucleic acid molecules are operatively linked to

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one or more transcription control sequences capable of being expressed in a mammalian cell.

118. The composition of Claim 103, wherein said transcription control sequences are selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR control sequences, SV-40 control sequences and β-actin control sequences.

119. The composition of Claim 103, wherein said nucleic acid molecule comprises a recombinant molecule selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST and PCR_3-GM_3.

120. The composition of Claim 103, wherein said therapeutic composition is useful for treating a cancer selected from the group consisting of melanomas, squamous cell carcinoma, breast cancers, head and neck carcinomas, thyroid carcinomas, soft tissue sarcomas, bone sarcomas, testicular cancers, prostatic cancers, ovarian cancers, bladder cancers, skin cancers, brain cancers, angiosarcomas, hemangiosarcomas, mast cell tumors, primary hepatic cancers, lung cancers, pancreatic cancers, gastrointestinal cancers, renal cell carcinomas, hematopoietic neoplasias, leukemias and lymphomas.

121. The composition of Claim 103, wherein said therapeutic composition is useful for treating a cancer selected from the group consisting of melanomas, lung cancers, thyroid carcinomas, breast cancers, renal cell

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carcinomas, squamous cell carcinomas, brain tumors and skin cancers.

122. A recombinant molecule comprising an isolated nucleic acid molecule encoding a superantigen and an isolated nucleic acid molecule encoding a cytokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

123. The molecule of Claim 122, wherein said recombinant molecule is capable being expressed in a mammalian cell.

124. The molecule of Claim 122, wherein said recombinant molecule contains a transcription control sequence selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR control sequences, SV-40 control sequences and β-actin control sequences.

125. The molecule of Claim 122, wherein said superantigen-encoding nucleic acid molecule encodes a toxin selected from the group consisting of staphylococcal enterotoxins, retroviral antigens, streptococcal antigens, mycoplasma antigens, mycobacteria antigens, virus antigens and protozoan antigens.

126. The molecule of Claim 122, wherein said superantigen-encoding nucleic acid molecule encodes a toxin selected from the group consisting of a SEA, SEB, SEC_1, SEC_2, SEC_3, SED, SEE and TSST gene.

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127. The molecule of Claim 122, wherein said superantigen-encoding nucleic acid molecule lacks a bacterial leader sequence.

128. The molecule of Claim 122, wherein said recombinant molecule is selected from the group consisting of PCR_3-SEB, PCR_3-SEA, PCR_3-SEB.S, PCR_3-SEA.S, PCR_3-TSST and PCR_3-GM_3.

129. A therapeutic composition comprising a delivery vehicle carrying an isolated nucleic acid molecule encoding a superantigen and an isolated nucleic acid molecule encoding a cytokine, wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences.

130. The composition of Claim 129, wherein said delivery vehicle is selected from the group consisting of a liposome, a micelle, a cell and a cellular membrane.

131. The composition of Claim 129, wherein said delivery vehicle comprises a liposome.

132. The composition of Claim 129, wherein said delivery vehicle comprises DOTMA and DOPE.

133. The composition of Claim 132, wherein said compound is a tumor cell ligand.

134. A method for increasing effector cell immunity in an animal, said method comprising administering to an animal an effective amount of a therapeutic composition comprising:

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a)   an isolated nucleic acid molecule encoding a superantigen; and

b)   an isolated nucleic acid molecule encoding a cytokine,

5   wherein said isolated nucleic acid molecules are operatively linked to one or more transcription control sequences, and wherein said therapeutic composition is targeted to a site in said animal that contains an abnormal cell.

10   135. The method of Claim 134, wherein said abnormal cell is selected from the group consisting of a cancer cell, a cell infected with an infectious agent and a non-cancerous cell having abnormal proliferative growth.

136. The method of Claim 134, wherein said abnormal

15   cell is a cancer cell.

137. The method of Claim 134, wherein said site is a tumor.

138. A method to suppress T cell activity in an animal, said method comprising administering to an animal

20   an effective amount of a therapeutic composition comprising:

a)   a naked isolated nucleic acid molecule encoding a superantigen; and

b)   a pharmaceutically acceptable carrier,

25   wherein said isolated nucleic acid molecule is operatively linked to a traynscription control sequence, and wherein said therapeutic composition is targeted to a

site in said animal that contains excessive T cell activity.

139. The method of Claim 138, wherein said composition is capable of suppressing said T cell activity for at least about 6 weeks in said animal.

140. The method of Claim 138, wherein said composition is capable of suppressing said T cell activity for at least about 8 weeks in said animal.

141. The method of Claim 138, wherein said composition is capable of suppressing said T cell activity for about 10 weeks in said animal.

142. The method of Claim 138, wherein said carrier comprises an aqueous physiologically balanced solution.

3 Different Bacterial Superantigens (SEB, SEA, TSST) Can Be Expressed in Eukaryotic Cells
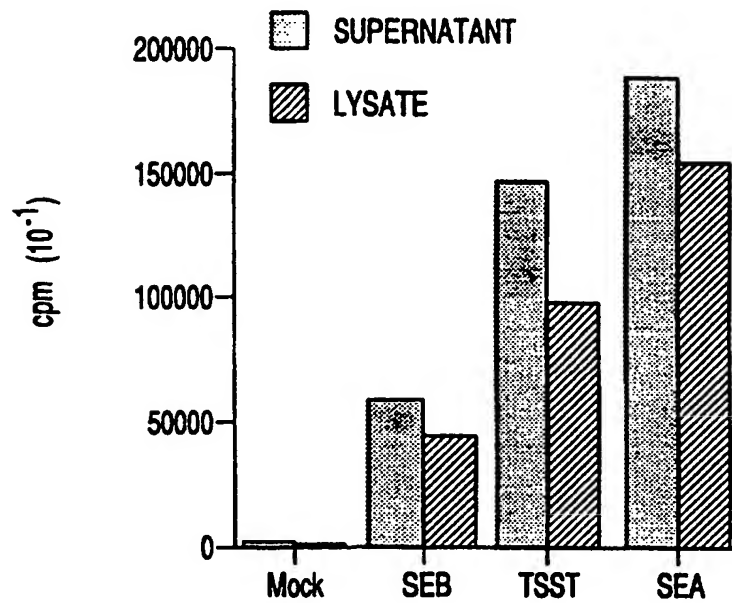
Fig. 1

Proliferative Response of Canine PBMC to SEB
Transfected Canine Melanoma Cells (MM4)
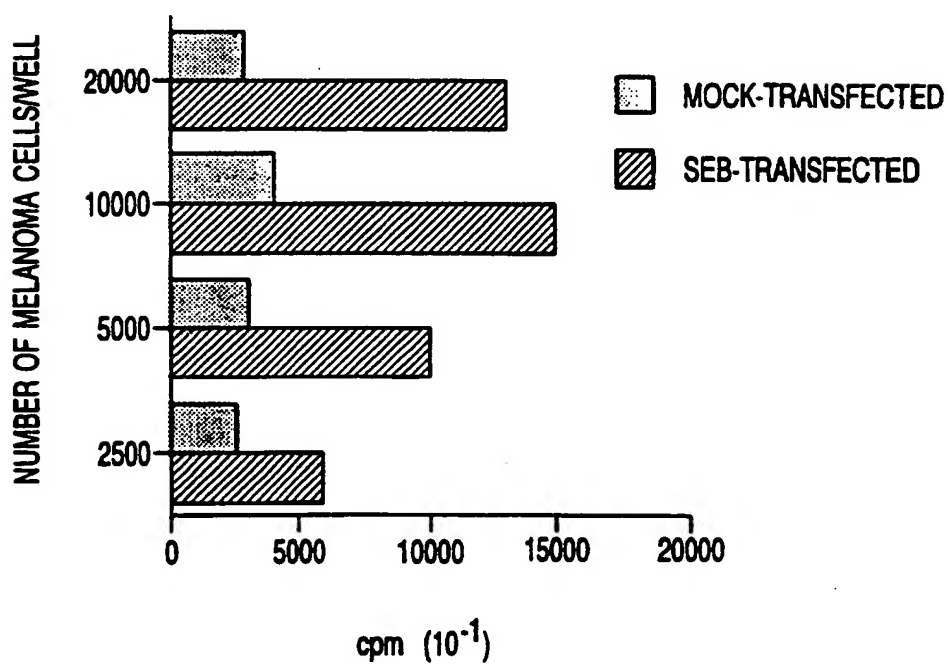
Fig. 2

Fig. 3A

SEA is Released from Stably Transfected CHO Cells
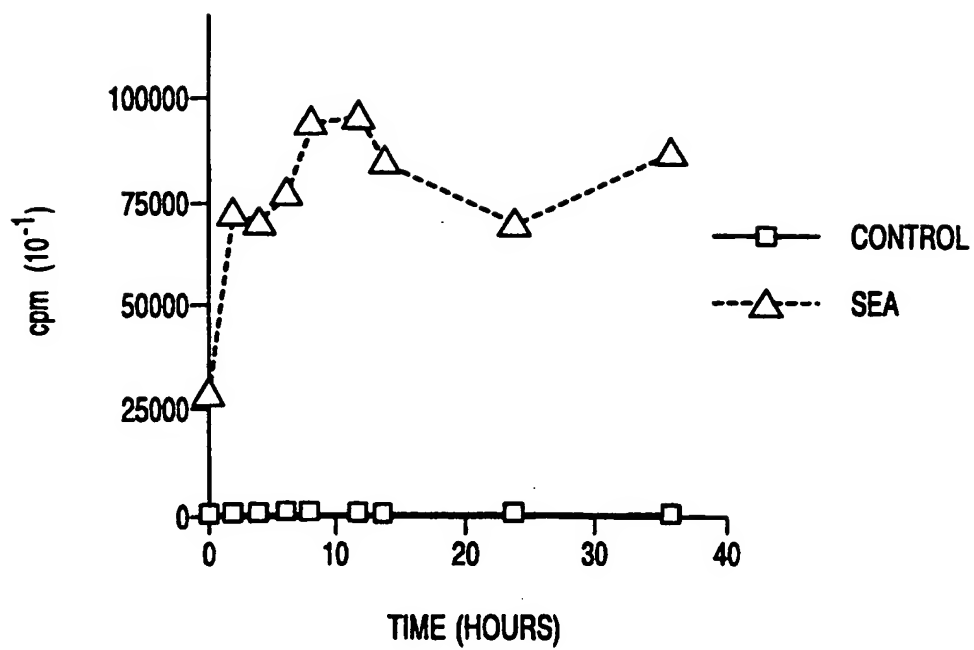
Fig. 3B

Proliferative Response of Vb3+ T Cell Clone (AD10) to SEA
Transfected B16 Melanoma Cells

cpm (10^{-1})
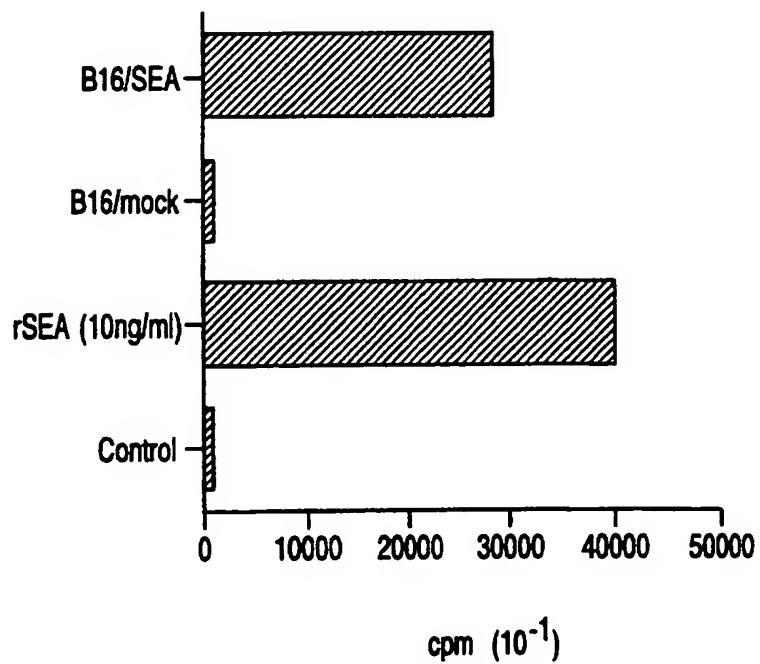
Fig. 4

Canine GM-CSF Activity in Supernatants
From Transfected CHO Cells
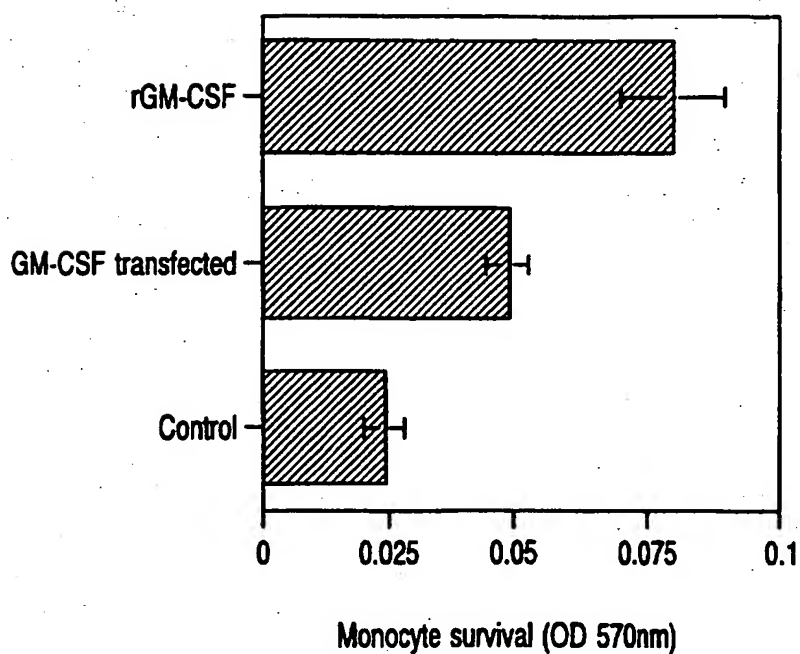
Fig. 5

Vaccination with SEB Transfected Tumor
Cells Generates Potent CTL Activity
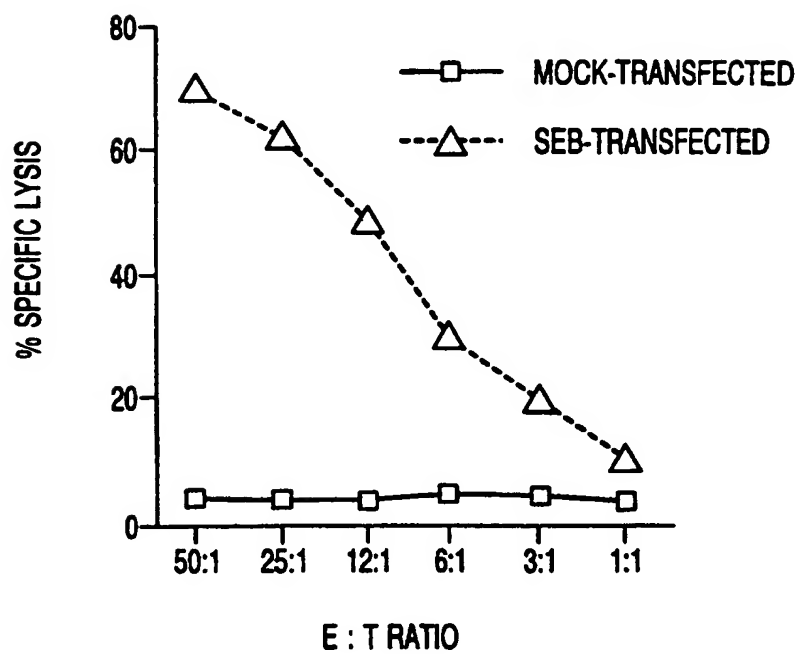
Fig. 6A

Vaccination with SEA Transfected Tumor Cells
Generates Potent CTL Activity
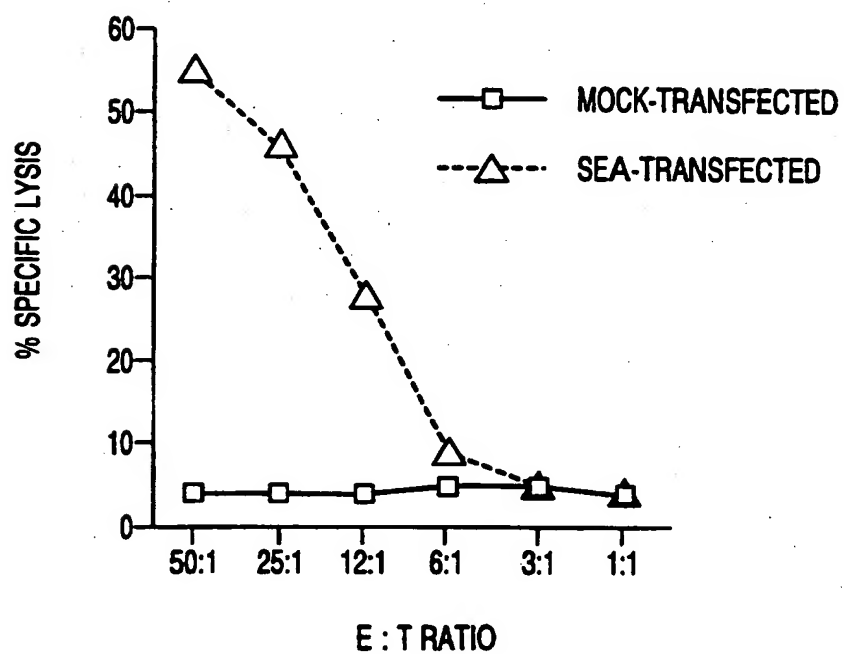
Fig. 6B

Effect of Tumor Target Transfection on CTL Lysis
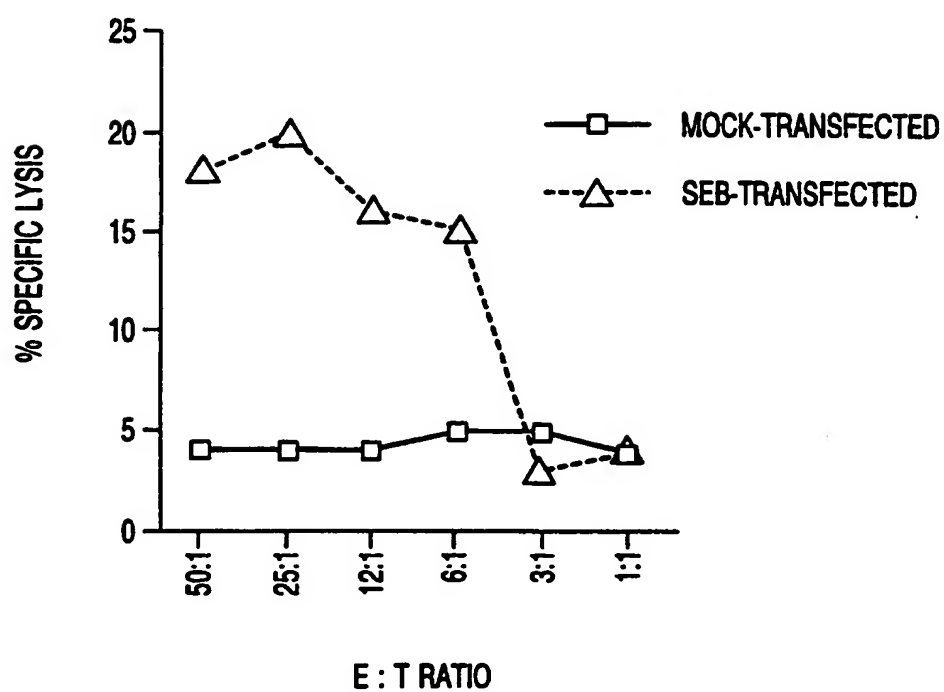

Fig. 7

Response of VB3+ T Cells to IM SEA/DNA Injection
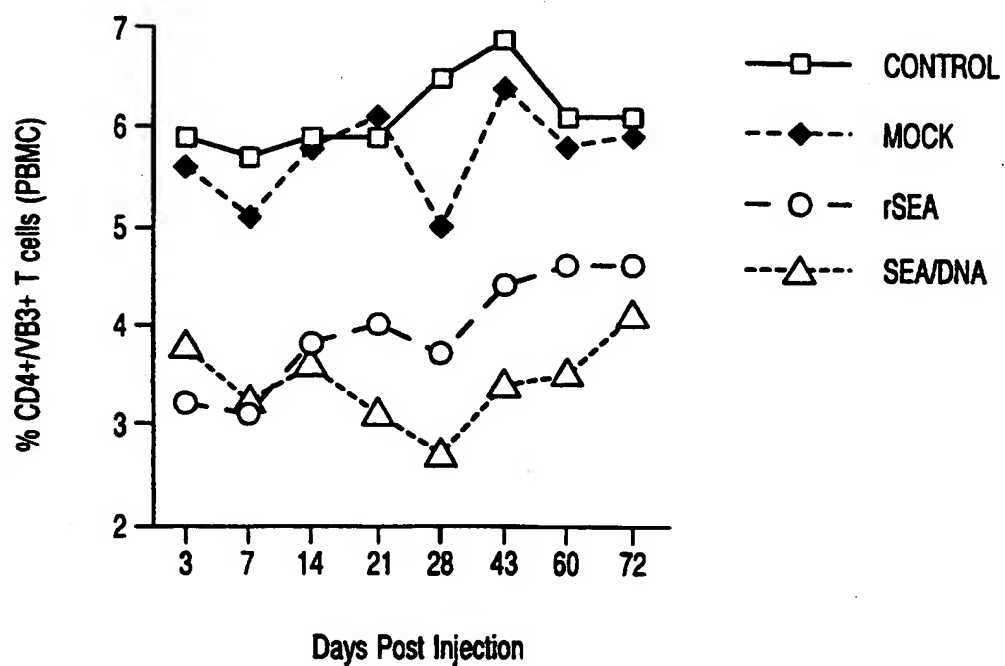
Fig. 8

Antibody Response to Single OVA DNA Injection (day 20)

antibody level (OD 410nm)
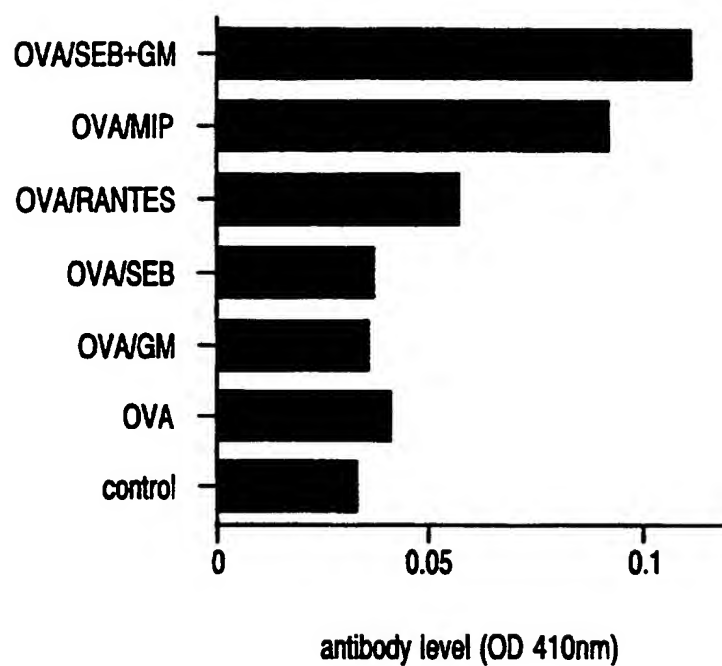
Fig. 9

Interferon Gamma Release after Stimulation with EG7-OVA

Fig. 10

Proliferative Response to EG7-OVA

Fig. 11

Fig. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/07432

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 48/00
US CL :514/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, BIOSIS, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95/00178 A1 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 05 January 1995, see entire document.	1-142
Y	DONNELLY et al. Protective Efficacy of Intramuscular Immunization with Naked DNA. Ann. N.Y. Acad. Sci. 1995, Vol.772, pages 40-46, see entire document.	1-142
Y	BLACKMAN et al. In Vivo Effects of Superantigens. Life Sciences. 1995, Vol.57, No.19, pages 1717-1735, see entire document.	1-142
Y	MIETHKE et al. Superantigen Mediated Shock: A Cytokine Release Syndrome. Immunobiol. 1993, Vol.189, pages 270-284, see entire document.	1-142

☒ Further documents are listed in the continuation of Box C.	☐ See patent family annex.

* Special categories of cited documents:

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 JULY 1996	23 AUG 1996

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer D. CURTIS HOGUE, JR.
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Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MIETHKE et al. Superantigens: The Paradox of T-Cell Activation versus Inactivation. Int. Arch. Allergy Immunol. 1995, Vol.106, pages 3-7, see entire document.	1-142
Y	LIU et al. Overview of DNA Vaccines. Ann. N.Y. Acad. Sci. 1995, Vol.772, pages 15-20, see entire document.	1-142

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